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- (71) Applicants (for all designated States except US): F. HOFFMANN-LA ROCHE AG [CH/CH]; Grenzacherstrasse 124, CH-4070 Basel (CH). MORPHOSYS AG [DE/DE]; Lena-Christ-Strasse 48, 82152 Martinsried (DE).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): BARDROFF, Michael [DE/DE]; Schietweg 2a, 81375 München (DE). BOHRMANN, Bernd [DE/DE]; Schlierbergstrasse 23, 79100 Freiburg (DE). BROCKHAUS, Manfred [DE/CH]; Talweg 29, CH-4126 Bettingen (CH). HUBER, Walter [CH/CH]; Ziegelhofweg 62, CH-4303 Kaiseraugst (CH). KRETZSCHMAR, Titus [DE/DE]; Pemmlerstrasse 10, 86857 Hurlach (DE). LÖHNING, Corinna [DE/DE]; Fleckhamerstrasse 12, 82131 Stockdorf (DE). LOETSCHER, Hansruedi [CH/CH]; Frankenstrasse

18, CH-4313 Möhlin (CH). **NORDSTEDT, Christer** [SE/SE]; Forskargatan 20, S-151 85 Sodertalje (SE). **ROTHE, Christine** [DE/DE]; Heinrich-Nicolausstrasse 26, 85221 Dachau (DE).

- (74) Agent: VOSSIUS & PARTNER; Siebertstrasse 4, 81675 Munich (DE).
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(54) Title: ANTI-Aβ ANTIBODIES AND THEIR USE

(57) Abstract: The present invention relates to antibody molecules capable of specifically recognizing two regions of the R-A4 peptide, wherein the first region comprises the amino acid sequence AEFRHDSGY as shown in SEQ ID NO: 1 or a fragment thereof and wherein the second region comprises the amino acid sequence VHHQKLVFFAEDVG as shown in SEQ ID NO: 2 or a fragment thereof. Furthermore, nucleic acid molecules encoding the inventive antibody molecules and vectors and hosts comprising said nucleic acid molecules are disclosed. In addition, the present invention provides for compositions, preferably pharmaceutical or diagnostic compositions, comprising the compounds of the invention as well as for specific uses of the antibody molecules, nucleic acid molecules, vectors or hosts of the invention.



### Anti-Aß antibodies and their use

The present invention relates to antibody molecules capable of specifically recognizing two regions of the  $\beta$ -A4 peptide, wherein the first region comprises the amino acid sequence AEFRHDSGY as shown in SEQ ID NO: 1 or a fragment thereof and wherein the second region comprises the amino acid sequence VHHQKLVFFAEDVG as shown in SEQ ID NO: 2 or a fragment thereof. Furthermore, nucleic acid molecules encoding the inventive antibody molecules and vectors and hosts comprising said nucleic acid molecules are disclosed. In addition, the present invention provides for compositions, preferably pharmaceutical or diagnostic compositions, comprising the compounds of the invention as well as for specific uses of the antibody molecules, nucleic acid molecules, vectors or hosts of the invention.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including any manufacturers specifications, instructions, etc.) are hereby incorporated by reference.

About 70% of all cases of dementia are due to Alzheimer's disease which is associated with selective damage of brain regions and neural circuits critical for cognition. Alzheimer's disease is characterized by neurofibrillary tangles in particular in pyramidal neurons of the hippocampus and numerous amyloid plaques containing mostly a dense core of amyloid deposits and defused halos.

The extracellular neuritic plaques contain large amounts of a pre-dominantly fibrillar peptide termed "amyloid  $\beta$ ", "A-beta", "A $\beta$ 4", " $\beta$ -A4" or "A $\beta$ "; see Selkoe (1994), Ann. Rev. Cell Biol. 10, 373-403, Koo (1999), PNAS Vol. 96, pp. 9989-9990, US

4,666,829 or Glenner (1984), BBRC 12, 1131. This amyloid  $\beta$  is derived from "Alzheimer precursor protein/ $\beta$ -amyloid precursor protein" (APP). APPs are integral membrane glycoproteins (see Sisodia (1992), PNAS Vol. 89, pp. 6075) and are endoproteolytically cleaved within the A $\beta$  sequence by a plasma membrane protease,  $\alpha$ -secretase (see Sisodia (1992), loc. cit.). Furthermore, further secretase activity, in particular  $\beta$ -secretase and  $\gamma$ -secretase activity leads to the extracellular release of amyloid- $\beta$  (A $\beta$ ) comprising either 39 amino acids (A $\beta$ 39), 40 amino acids (A $\beta$ 40), 42 amino acids (A $\beta$ 42) or 43 amino acids (A $\beta$ 43); see Sinha (1999), PNAS 96, 11094-1053; Price (1998), Science 282, 1078 to 1083; WO 00/72880 or Hardy (1997), TINS 20, 154.

It is of note that  $A\beta$  has several naturally occurring forms, whereby the human forms are referred to as the above mentioned  $A\beta39$ ,  $A\beta40$ ,  $A\beta41$ ,  $A\beta42$  and  $A\beta43$ . The most prominent form,  $A\beta42$ , has the amino acid sequence (starting from the N-terminus): DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA (SEQ ID NO: 27). In  $A\beta41$ ,  $A\beta40$ ,  $A\beta39$ , the C-terminal amino acids A, IA and VIA are missing, respectively. In the  $A\beta43$ -form an additional threonine residue is comprised at the C-terminus of the above depicted sequence (SEQ ID NO: 27).

The time required to nucleate A $\beta$ 40 fibrils was shown to be significantly longer than that to nucleate A $\beta$ 42 fibrils; see Koo, loc. cit. and Harper (1997), Ann. Rev. Biochem. 66, 385-407. As reviewed in Wagner (1999), J. Clin. Invest. 104, 1239-1332, the A $\beta$ 42 is more frequently found associated with neuritic plaques and is considered to be more fibrillogenic in vitro. It was also suggested that A $\beta$ 42 serves as a "seed" in the nucleation-dependent polymerization of ordered non-crystalline A $\beta$  peptides; Jarrett (1993), Cell 93, 1055-1058.

It has to be stressed that modified APP processing and/or the generation of extracellular plaques containing proteinaceous depositions are not only known from Alzheimer's pathology but also from subjects suffering from other neurological and/or neurodegenerative disorders. These disorders comprise, inter alia, Down's syndrome, Hereditary cerebral hemorrhage with amyloidosis Dutch type, Parkinson's

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disease, ALS (amyotrophic lateral sclerosis), Creutzfeld Jacob disease, HIV-related dementia and motor neuropathy.

In order to prevent, treat and/or ameliorate disorders and/or diseases related to the pathological deposition of amyloid plaques, means and methods have to be developed which either interfere with  $\beta$ -amyloid plaque formation, which are capable of preventing  $A\beta$  aggregation and/or are useful in de-polymerization of already formed amyloid deposits or amyloid- $\beta$  aggregates.

Accordingly, and considering the severe defects of modified and/or pathological amyloid biology, means and methods for treating amyloid related disorders are highly desirable. In particular, efficient drugs which either interfere with pathological amyloid aggregation or which are capable of de-polymerization of aggregated  $A\beta$  are desired. Furthermore, diagnostic means are desirable to detect, inter alia, amyloid plaques.

Thus, the technical problem of the present invention is to comply with the needs described herein above.

Accordingly, the present invention relates to an antibody molecule capable of specifically recognizing two regions of the  $\beta$ -A4/A $\beta$ 4 peptide, wherein the first region comprises the amino acid sequence AEFRHDSGY (SEQ ID NO: 1) or a fragment thereof and wherein the second region comprises the amino acid sequence VHHQKLVFFAEDVG (SEQ ID NO: 2) or a fragment thereof.

In context of the present invention, the term "antibody molecule" relates to full immunoglobulin molecules, preferably IgMs, IgDs, IgEs, IgAs or IgGs, more preferably IgG1, IgG2a, IgG2b, IgG3 or IgG4 as well as to parts of such immunoglobulin molecules, like Fab-fragments or  $V_{L^-}$ ,  $V_{H^-}$  or CDR-regions. Furthermore, the term relates to modified and/or altered antibody molecules, like chimeric and humanized antibodies. The term also relates to modified or altered monoclonal or polyclonal antibodies as well as to recombinantly or synthetically generated/synthesized antibodies. The term also relates to intact antibodies as well

as to antibody fragments/parts thereof, like, separated light and heavy chains, Fab, Fab/c, Fv, Fab', F(ab')<sub>2</sub>. The term "antibody molecule" also comprises antibody derivatives, the bifunctional antibodies and antibody constructs, like single chain Fvs (scFv), bispecific scFvs or antibody-fusion proteins. Further details on the term "antibody molecule" of the invention are provided herein below.

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The term "specifically recognizing" means in accordance with this invention that the antibody molecule is capable of specifically interacting with and/or binding to at least two amino acids of each of the two regions of  $\beta$ -A4 as defined herein. Said term relates to the specificity of the antibody molecule, i.e. to its ability to discriminate between the specific regions of the  $\beta$ -A4 peptide as defined herein and another, not another. APP-related or not peptide **β-A4** region of the related protein/peptide/(unrelated) tests-peptide. Accordingly, specificity can be determined experimentally by methods known in the art and methods as disclosed and described herein. Such methods comprise, but are not limited to Western blots, ELISA-, RIA-, ECL-, IRMA-tests and peptide scans. Such methods also comprise the determination of  $K_D$ -values as, inter alia, illustrated in the appended examples. The peptide scan (pepspot assay) is routinely employed to map linear epitopes in a polypeptide antigen. The primary sequence of the polypeptide is synthesized successively on activated cellulose with peptides overlapping one another. The recognition of certain peptides by the antibody to be tested for its ability to detect or recognize a specific antigen/epitope is scored by routine colour development (secondary antibody with horseradish peroxidase and 4-chloronaphthol and hydrogenperoxide), by a chemoluminescence reaction or similar means known in the art. In the case of, inter alia, chemoluminescence reactions, the reaction can be quantified. If the antibody reacts with a certain set of overlapping peptides one can deduce the minimum sequence of amino acids that are necessary for reaction; see illustrative Example 6 and appended Table 2.

The same assay can reveal two distant clusters of reactive peptides, which indicate the recognition of a discontinuous, i. e. conformational epitope in the antigenic polypeptide (Geysen (1986), Mol. Immunol. 23, 709-715).

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In addition to the pepspot assay, standard ELISA assay can be carried out. As demonstrated in the appended examples small hexapeptides may be coupled to a protein and coated to an immunoplate and reacted with antibodies to be tested. The scoring may be carried out by standard colour development (e.g. secondary antibody with horseradish peroxidase and tetramethyl benzidine with hydrogenperoxide). The reaction in certain wells is scored by the optical density, for example at 450 nm. Typical background (=negative reaction) may be 0.1 OD, typical positive reaction may be 1 OD. This means the difference (ratio) positive/negative can be more than 10 fold. Further details are given in the appended examples. Additional, quantitative methods for determining the specificity and the ability of "specifically recognizing" the herein defined two regions of the  $\beta$ -A4 peptide are given herein below.

The term "two regions of the  $\beta$ -A4 peptide" relates to two regions as defined by their amino acid sequences shown in SEQ ID NOs: 1 and 2, relating to the N-terminal amino acids 2 to 10 and to the central amino acids 12 to 25 of  $\beta$ -A4 peptide. The term "β-A4 peptide" in context of this invention relates to the herein above described A $\beta$ 39, A $\beta$ 41, A $\beta$ 43, preferably to A $\beta$ 40 and A $\beta$ 42. A $\beta$ 42 is also depicted in appended SEQ ID NO: 27. It is of note that the term "two regions of the  $\beta$ -A4 peptide" also relates to an "epitope" and/or an "antigenic determinant" which comprises the herein defined two regions of the  $\beta$ -A4 peptide or parts thereof. In accordance with this invention, said two regions of the  $\beta$ -A4 peptide are separated (on the level of the amino acid sequence) in the primary structure of the  $\beta$ -A4 peptide by at least one amino acid, preferably by at least two amino acids, more preferably by at least three amino acids, more preferably by at least four amino acids, more preferably by at least five amino acids, more preferably at least six amino acids, more preferably at least nine amino acids and most preferably at least twelve amino acids. As shown documented in the appended examples, the and as herein antibodies/antibody molecules detect/interact with and/or bind to two regions of the  $\beta$ -A4 peptide as defined herein, whereby said two regions are separated (on the primary structure level of the amino acid sequence) by at least one amino acid and wherein the sequence separating said two regions/"epitope" may comprise more then ten amino acids, preferably 14 amino acids, more preferably 15 amino acids or

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16 amino acids. For example, MSR-3 Fab (as an inventive antibody molecule) recognizes detects/interacts with two regions on the β-A4 peptide, wherein said first region comprises amino acids 3 and 4 (EF) and said second regions comprises amino acids 18 to 23 (VFFAED). Accordingly, the separating sequence between the region/epitopes to be detected/recognized has a length of 13 amino acids on the primary amino acid sequence structure. Similarly, MSR #3.4H7 IgG1, an optimized and matured antibody molecules derived from MSR-3 and comprised in an IgG1framework, detects/interacts with/binds to two epitopes/regions of β-A4 which comprise in the first region positions 1 to 4 (DAEF) and in the second region positions 19 to 24 (FFAEDV) of β-A4 as defined herein. Accordingly, MSR #3.4H7 IgG1 recognizes/detects/interacts with/binds to two epitopes/regions which are, on the primary amino acid sequence level, separated by 14 amino acids. As detailed in the appended examples, affinity maturation and conversion of monovalent inventive Fab fragments to full-length IgG1 antibodies may result in a certain broadening of the epitopes/regions detected in pepspot, ELISA assays and the like. Therefore, the antibody molecules of the invention are capable of simultaneously and independently recognizing two regions of the  $\beta$ -A4 peptide/A $\beta$ 4 wherein said regions comprise the amino acid sequence as shown in SEQ ID NO: 1 (or parts thereof) and the amino acid sequence as shown in SEQ ID NO: 2 (or (a) part(s) thereof). Due to the potential broadening of epitopes as detailed herein it is, however, also envisaged that amino acids in close proximity to the sequences of SEQ ID NO: 1 and 2 are detected/recognized, i.e. that additional amino acids are part of the two regions to be detected/recognized. Accordingly, it is also envisaged that, e.g. the first amino acid of Aβ (1-42) as defined herein, namely D (Aspartic acid) in part of one epitope to be detected/recognized or that amino acids located after the region of AB (1-42) as defined in SEQ ID NO: 2 are detected/recognized. Said additional amino acid may, e.g., be the amino acid on position 26 of SEQ ID NO: 27 (βA4/Aβ (1-42)), namely S (Serine).

The term may also relate to a conformational epitope, a structural epitope or a discountinuous epitope consisting of said two regions or parts thereof; see also Geysen (1986), loc. cit. In context of this invention, a conformational epitope is defined by two or more discrete amino acid sequences separated in the primary

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sequence which come together on the surface when the polypeptide folds to the native protein (Sela, (1969) Science 166, 1365 and Laver, (1990) Cell 61, 553-6). The antibody molecules of the present invention are envisaged to specifically bind to/interact with a conformational/structural epitope(s) composed of and/or comprising the two regions of  $\beta$ -A4 described herein or parts thereof as disclosed herein below. The "antibody molecules" of the present invention are thought to comprise a simultaneous and independent dual specificity to (a) an amino acid stretch comprising amino acids 2 to 10 (or (a) part(s) thereof) of  $\beta$ -A4 and (b) an amino acid stretch comprising amino acids 12 to 25 (or (a) part(s) thereof) of  $\beta$ -A4 (SEQ ID NO. 27). Fragments or parts of these stretches comprise at least two, more preferably at least three amino acids. Preferred fragments or parts are in the first region/stretch of SEQ ID NO: 27 the amino acid sequences AEFRHD, EF, EFR, FR, EFRHDSG, EFRHD or HDSG and in the second region/stretch of SEQ ID NO: 27 the amino acid sequences HHQKL, LV, LVFFAE, VFFAED VFFA, or FFAEDV. As mentioned above, said fragments may also comprise additional amino acids or may be parts of the fragments defined herein. Specific examples are DAE, DAEF, FRH or RHDSG.

A number of antibodies specifically recognizing A $\beta$  peptides have been described in the art. These antibodies have mainly been obtained by immunizing animals with A $\beta$ 1-40 or A $\beta$ 1-42 or fragments thereof using standard technologies. According to published data monoclonal antibodies that were generated by immunization with the complete A $\beta$  peptide (1-40 or 1-42) recognize exclusively an epitope close to the N-terminus of A $\beta$ . Further, examples are the antibodies BAP-1 and BAP-2 (Brockhaus, unpublished) which were generated by immunization of mice with A $\beta$ 1-40 and which recognize the amino acids 4-6 in the context of larger A $\beta$  peptides; see appended Example 7, Table 2 and Example 12, Table 7. Antibodies that recognize the middle part of A $\beta$  derive from immunizations with smaller peptides. For example, the antibody 4G8 was generated by immunization with the A $\beta$  peptide 1-24 and recognizes exclusively the sequence 17-24 (Kim, (1988) Neuroscience Research Communications 2, 121-130). Many other monoclonal antibodies have been generated by immunizing mice with A $\beta$ -derived fragments, and antibodies recognizing the C-terminal end of A $\beta$ 1-40 and A $\beta$ 1-42 are widely used to distinguish

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and quantitate the corresponding A $\beta$  peptides in biological fluids and tissues by ELISA, Western blot and immunohistochemistry analysis (Ida et al, (1996) J. Biol. Chem. 271, 22908-22914; Johnson-Wood et al., (1997), Proc. Natl. Acad. Sci. USA (1994), 1550-1555; Suzuki et al., (1994), Science 264, 1336-1340; Brockhaus (1998), Neuro Rep. 9, 1481-1486). BAP-17 is a mouse monoclonal antibody which has been generated by immunizing mice with A $\beta$  fragment 35-40. It specifically recognizes the C-terminal end of A $\beta$ 1-40 (Brockhaus (1998) Neuroreport 9, 1481-1486).

It is believed that the immunization with T-cell dependent antigens (often poor immunogens) requires a proteolytic cleavage of the antigen in the endosomes of antigen presenting cells. The in vivo selection of high affinity antibodies after immunization is driven by the contact of helper T cells to antigen presenting cells. The antigen presenting cells only present short peptides and not polypeptides of large size. Accordingly, these cells have a complicated (but well known) machinery to endocytose antigen(s), degrade the antigen(s) in endosomes, combine selected peptides with suitable MHC class II molecules, and to export the peptide-MHC complex to the cell surface. This is where the antigen specific recognition by T cells occurs, with the aim to provide help to maturing B cells. The B cells which receive most T cell help have the best chance to develop into antibody secreting cells and to proliferate. This shows that antigen processing by proteolysis is an important step for the generation of an high affinity antibody response in vivo and may explain the dominance of the N-terminal A $\beta$  epitope in prior art monoclonal and polyclonal antibodies derived by immunization.

In contrast, the selection of antibodies/antibody molecules of the present invention is driven by the physical adherence of Fab expressing phages to the antigen. There is no degradation of the antigen involved in this in vitro selection process. The phages which express the Fab with the highest affinity towards the antigen are selected and propagated. A synthetic library as employed in the appended examples to select for specific antibody molecules according to this invention is particularly suited for avoiding any bias for single, continuous epitopes that is often found in libraries derived from immunized B cells.

It is of note that the prior art has not described antibody molecules recognizing two, independent regions of A $\beta$ 4 which specifically recognizes (a) discontinuous/structural/conformational epitope(s) and/or which are capable of simultaneously and independently recognizing two regions/epitopes of A $\beta$ 4.

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Vaccination of transgenic mice overexpressing mutant human APP V717F (PDAPP mice) with Aβ1-42 resulted in an almost complete prevention of amyloid deposition in the brain when treatment was initiated in young animals, i. e. before the onset of neuropathologies, whereas in older animals a reduction of already formed plaques was observed suggesting antibody-mediated clearance of plaques (Schenk et al., (1999). Nature 400,173-177). The antibodies generated by this immunization procedure were reactive against the N-terminus of Aβ4 covering an epitope around amino acids 3-7 (Schenk et al., (1999), loc. cit.; WO 00/72880). Active immunization with Aβ1-42 also reduced behavioural impairment and memory loss in different transgenic models for Alzheimer's Disease (Janus et al., (2000) Nature 408, 979-982; Morgan et al., (2000) Nature 408, 982-985). Subsequent studies with peripherally administered antibodies, i. e. passive immunization, have confirmed that antibodies can enter the central nervous system, decorate plagues and induce clearance of preexisting amyloid plaques in APP transgenic mice (PDAPP mice) (Bard et al., (2000) Nat. Med. 6, 916-919; WO 00/72880). In these studies, the monoclonal antibodies with the most potent in vivo and ex vivo efficacy (triggering of phagocytosis in exogenous microglial cells) were those which recognized Aβ4 Nterminal epitopes 1-5 (mab 3D6, IgG2b) or 3-6 (mab 10D5, IgG1). polyclonal antibodies isolated from mice, rabbits or monkeys after immunization with Aß1-42 displayed a similar N-terminal epitope specificity and were also efficacious in triggering phagocytosis and in vivo plaque clearing. In contrast, C-terminal specific antibodies binding to A\u00e31-40 or A\u00e31-42 with high affinity did not induce phagocytosis in the ex vivo assay and were not efficacious in vivo (WO 00/72880). Monoclonal antibody m266 (WO 00/72880) was raised against A $\beta$ 13-28 (central domain of A $\beta$ ) and epitope mapping confirmed the antibody specificity to cover amino acids 16-24 in the  $A\beta$  sequence. This antibody does not bind well to aggregated  $A\beta$  and amyloid deposits and merely reacts with soluble (monomeric) AB, i. e. properties which are similar to another well-known and commercially available monoclonal antibody (4G8;

Kim, (1988) Neuroscience Research Communications 2, 121-130; commercially available from Signet Laboratories Inc. Dedham, MA USA) which recognizes the same epitope.

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In vivo, the m266 antibody was recently found to markedly reduce A $\beta$  deposition in PDAPP mice after peripheral administration (DeMattos, (2001) Proc. Natl. Acad. Sci. USA 98, 8850-8855). However, and in contrast to N-terminal specific antibodies, m266 did not decorate amyloid plaques *in vivo*, and it was therefore hyothesized that the brain A $\beta$  burden was reduced by an antibody-induced shift in equilibrium between CNS and plasma A $\beta$  resulting in the accumulation of brain-derived A $\beta$  in the periphery, firmly complexed to m266 (DeMattos, (2001) loc. cit.).

The antibodies/antibody molecules of the present invention, by simultaneously (for example in a structural/conformational epitope formed by the N-terminal and central region of ßA4 as described herein) and independently (for example in pepspot assays as documented in the appended experimental part) binding to the N-terminal and central epitopes, combine the properties of an N-terminal-specific antibody and a central epitope-specific antibody in a single molecule. Antibodies with the dual epitope specificity, as described in the present invention, are considered to be more efficacious in vivo, in particular in medical and diagnostic settings for, e.g., reducing amyloid plaque burden or amyloidogenesis or for the detection of amyloid deposits and plaques. It is well known that in the process of AB4 aggregation and amyloid deposition conformational changes occur, and while the central epitope is easily accessible in soluble Aβ4 it appears to be hidden and less reactive in aggregated or fibrillar Aβ4. The fact that the central/middle epitope-specific antibody m266 is efficacious in vivo indicates that neutralization of soluble Aβ4 may also be a critical parameter. The antibodies/antibody molecules of the present invention, due to the dual epitope specificity, can bind to both fibrillar and soluble A\beta 4 with similar efficacy, thus allowing interaction with amyloid plaques as well as neutralization of soluble Aβ4. The term "simultaneously and independently binding to the N-terminal and central/middle epitopes of β-A4" as employed herein in context of the inventive antibody molecules relates to the fact that the antibodies/antibody molecules

described herein may detect and/or bind to both epitopes simultaneously, i.e. at the same time (for example on conformational/structural epitopes formed by the N-terminal epitope (or (a) part(s) thereof) and central epitopes (or (a) part(s) thereof) of ßA4 as defined herein) and that the same antibody molecules, however, are also capable of detecting/binding to each of the defined epitopes in an independent fashion, as inter alia, demonstrated in the pepspot analysis shown in the examples.

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Clearance of amyloid plaques *in vivo* in PDAPP mice after direct application of the antibodies to the brain is not dependent on the IgG subtype and may also involve a mechanism which is not Fc-mediated, i. e. no involvement of activated microglia in plaque clearance (Bacskai, (2001), Abstract Society for Neuroscience 31<sup>st</sup> Annual Meeting, November 10-15, 2001, San Diego). This observation is in contrast to what has been postulated in an earlier study by Bard (2000), loc. cit.

In another study antibodies raised against A $\beta$ 1-28 and A $\beta$ 1-16 peptides were found to be effective in disaggregating A $\beta$  fibrils *in vitro*, whereas an antibody specific for A $\beta$ 13-28 was much less active in this assay (Solomon, (1997) Proc. Natl. Acad. Sci. USA 94, 4109-4112). Prevention of A $\beta$  aggregation by an anti-A $\beta$ 1-28 antibody (AMY-33) has also been reported (Solomon, (1996) Proc. Natl. Acad. Sci. USA 93, 452-455). In the same study, antibody 6F/3D which has been raised against A $\beta$  fragment 8-17 slightly interfered with Zn<sup>2+</sup>-induced A $\beta$  aggregation but had no effect on the self aggregation induced by other aggregation-inducing agents.

The efficacy of the various antibodies in these in vitro assays correlates with the accessibility of their epitopes in A $\beta$ 4 aggregates. The N-terminus is exposed and N-terminal specific antibodies clearly induce de-polymerization, whereas the central region and the C-terminus are hidden and not easily accessible and thus antibodies against these epitope are much less effective.

Investigations with respect to epitope accessibilty for antibodies have shown that in aggregated  $A\beta$  the N-terminal epitope is exposed and reacts with the BAP-1 antibody, whereas the middle or central epitope indeed remains cryptic, i. e. no binding of the 4G8 antibody was observed. However, in monomeric  $A\beta$  both epitopes are overt and are equally recognized by both prior art antibodies.

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In contrast, in the present invention, it was surprisingly found that the herein described antibody molecules recognize two discontinuous amino acid sequences, e.g. a conformational/structural epitope on the A $\beta$  peptide. Two "discontinuous amino acid sequences" in accordance with this invention means that said two amino acid sequences forming the N-terminal and central/middle epitopes, respectively, are separated on  $\beta$ -A4 in its primary structure by at least two amino acids which are not part of either epitope.

The binding area of an antibody Fab (=paratope) occupies a molecular surface of approximately 30 x 30 Å in size (Laver, Cell 61 (1990), 553-556). This is enough to contact 15 to 22 amino acid residues which may be present on several surface loops. The discontinuous epitope recognized by the inventive antibody molecules resembles a conformation in which the N-terminal (residues 2 to 10 or parts thereof) and middle  $A\beta$  peptide sequences (residues 12 to 25 or parts thereof) are in close proximity. Only within this conformation, the maximum number of antigen-antibody contacts and the lowest free energy state are obtained.

Based on energetic calculations it has been suggested that a smaller subset of 5-6 residues, which are not arranged in a linear sequence but are scattered over the epitope surface, contributes most of the binding energy while surrounding residues may merely constitute a complementary array (Laver (1990) loc. cit.).

The inventive antibodies/antibody molecules are capable of binding to aggregated  $A\beta$  and strongly react with amyloid plaques in the brain of AD patients (as documented in the appended examples). In addition, they are capable of depolymerizing/disintegrating amyloid aggregates.

Without being bound by theory, the conformational/structural epitope (composed by the two regions of A $\beta$ 4 or (a) part(s) of said regions as described herein) is believed to be partially exposed in aggregated A $\beta$ . However, it is known that major part of the middle/second epitope/region alone is not freely accessible in these A $\beta$  aggregates (based on the poor reactivities of middle epitope-specific antibodies 4G8 and m266). On the other hand, and in view of the considerations mentioned above, it is likely

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that one or several residues of the middle region are components of the conformational epitope and, in conjunction with the residues from the N-terminal region, are accessible to the antibodies of the present invention, thereby significantly contributing to the binding energy of the antibody-A $\beta$ 4 interaction. The reactivity of the inventive antibody molecules with the conformational epitope in aggregated A $\beta$  is therefore unique and clearly distinct from  $\alpha$ -A $\beta$ 4 antibodies described in the prior art. Yet, as pointed out herein above, a further unique feature of the inventive antibodies/antibody molecules is their capacity to simultaneously and independently binding to/recognizing two separate epitopes on  $\beta$ -A4, as defined herein and in the appended examples.

In a preferred embodiment of the invention, the inventive antibody molecule is an antibody molecule wherein the least two regions of the  $\beta$ -A4 to be specifically recognized by said antibody form a conformational/structural epitope or a discontinuous epitope; see Geysen (1986), loc. cit.; Ghoshal (2001), J. Neurochem. 77, 1372-1385; Hochleitner (2000), J. Imm. 164, 4156-4161; Laver (1990), loc. cit... The term "discontinuous epitope" means in context of the invention non-linear epitopes that are assembled from residues from distant portions of the polypeptide chain. These residues come together on the surface when the polypeptide chain folds into a three-dimensional structure to constitute a conformational/structural epitope. The present invention provides for preferred, unexpected epitopes within β-A4, which result in the inventive generation of specific antibody molecules, capable of specifically interacting with these epitopes. These inventive antibodies/antibody molecules provide the basis for increased efficacy, and a reduced potential for side effects. As pointed out above, the inventive antibodies, however, were also capable of independently interacting with each of the defined two regions/epitopes of  $\beta$ -A4, for example in Pepspot assays as documented in the appended examples.

The present invention, accordingly, provides for unique tools which may be employed to de-polymerize aggregated A $\beta$ -fibrils in vivo and in vitro and/or which are capable of stabilizing and/or neutralizing a conformational epitope of monomeric A $\beta$  and thereby capable of preventing the pathological A $\beta$  aggregation.

It is furthermore envisaged that the inventive antibodies bind to  $A\beta$  deposits at the rim of amyloid plaques in, inter alia, Alzheimer's brain and efficiently dissolve the pathological protofibrils and fibrils.

In a preferred embodiment, the antibody molecule of the invention recognizes at least two consecutive amino acids within the two regions of Aβ4 defined herein, more preferably said antibody molecule recognizes in the first region an amino acid sequence comprising the amino acids: AEFRHD, EF, EFR, FR, EFRHDSG, EFRHD or HDSG and in the second region an amino acid sequence comprising the amino acids: HHQKL, LV, LVFFAE, VFFAED, VFFA or FFAEDV. Further fragments or broadened parts comprise: DAE, DAEF, FRH or RHDSG.

It is particularly preferred that the antibody molecule of the invention comprises a variable V<sub>H</sub>-region as encoded by a nucleic acid molecule as shown in SEQ ID NO: 3, 5 or 7 or a variable V<sub>H</sub>-region as shown in the amino acid sequences depicted in SEQ ID NOs: 4, 6 or 8. The sequences as shown in SEQ ID NOs: 3 and 4 depict the coding region and the amino acid sequence, respectively, of the V<sub>H</sub>-region of the inventive, parental antibody MSR-3 (MS-Roche 3), the sequences in SEQ ID NOs: 5 and 6 depict the coding region and the amino acid sequence, respectively, of the V<sub>H</sub>region of the inventive, parental antibody MSR-7 (MS-Roche 7) and SEQ ID NOs: 7 and 8 depict the coding region and the amino acid sequence, respectively, of the V<sub>H</sub>region of the inventive, parental antibody MSR-8 (MS-Roche 8). Accordingly, the invention also provides for antibody molecules which comprise a variable V<sub>1</sub>-region as encoded by a nucleic acid molecule as shown in a SEQ ID NO selected from the group consisting of SEQ ID NO: 9, 11 or 13 or a variable V<sub>L</sub>-region as shown in the amino acid sequences depicted in SEQ ID NOs: 10, 12 or 14. SEQ ID NOs: 9 and 10 correspond to the V<sub>L</sub>-region of MSR-3, SEQ ID NOs: 11 and 12 correspond to the V<sub>L</sub>-region of MSR-7 and SEQ ID NOs: 13 and 14 correspond to the V<sub>L</sub>-region of MSR-8. As illustrated in the appended examples, the parental antibodies MSR-3, -7 and -8, are employed to further generate optimized antibody molecules with even better properties and/or binding affinities. Some of the corresponding and possible strategies are exemplified and shown in the appended examples.

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The optimization strategy as illustrated in the appended examples lead to a plurality of inventive, optimized antibodies. These optimized antibodies share with their parental antibodies the CDR-3 domain of the V<sub>H</sub>-region. Whereas the original framework region (as shown in appended Figure 1) remains the same, in the matured/optimized antibody molecules, CDR1, CDR2 and/or V<sub>L</sub> CDR3-regions are changed. Illustrative, modified sequence motives for optimized antibody molecules are shown in appended table 1. Accordingly, within the scope of the present invention are also optimized antibody molecules which are derived from the herein disclosed MSR-3, -7 and -8 and which are capable of specifically reacting with/specifically recognizing the two regions of the β-A4 peptide as defined herein. In particular, CDR-regions, preferably CDR1s, more preferably CDR1s and CDR2s, most preferably CDR1s, CDR2s and CDR3s as defined herein may be employed to generate further inventive antibodies/antibody molecules, inter alia, by CDR-grafting methods known in the art; see Jones (1986), Nature 321, 522-515 or Riechmann (1988), Nature 332, 323-327. Most preferably the inventive antibodies/antibody molecules as well as antibody fragments or derivatives are derived from the parental antibodies as disclosed herein and share, as disclosed above, the CDR-3 domain of the V<sub>H</sub>-region with at least one of said parental antibodies. As illustrated below, it is also envisaged that cross-cloned antibodies are generated which are to be considered as optimized/maturated antibodies/antibody molecules of the present invention. Accordingly, preferred antibody molecules may also comprise or may also be derived from antibodies/antibody molecules which are characterized by V<sub>H</sub>regions as shown in any of SEQ ID NOs: 32 to 45 or V<sub>L</sub>-regions as shown in SEQ ID NOs: 46 to 59 or which may comprise a CDR-3 region as defined in any of SEQ ID NOs: 60 to 87. In a particular preferred embodiment, the optimized antibody molecule of the present invention comprises V<sub>H</sub>-regions and V<sub>L</sub>-regions as depicted in SEQ ID NOs: 88/89 and 90/91, respectively, or parts thereof. Apart thereof may be (a) CDR-region(s), preferably (a) CDR3-region(s). A particularly preferred antibody molecule of the optimized type comprises a H-CDR3 as characterized in SEQ ID NOs: 92 or 93 and/or a L-CDR3 as characterized in SEQ ID NOs: 94 or 95. It is preferred that the antibodies/antibody molecules of the invention are characterized by their specific reactivity with β-A4 and/or peptides derived from said β-A4. For example, optical densities in ELISA-tests, as illustrated in the appended

examples, may be established and the ratio of optical densities may be employed to define the specific reactivity of the parental or the optimized antibodies. Accordingly, a preferred antibody of the invention is an antibody which reacts in an ELISA-test with  $\beta$ -A4 to arrive at an optical density measured at 450 nm that is 10 times higher than the optical density measured without  $\beta$ -A4, i. e. 10 times over background. Preferably the measurement of the optical density is performed a few minutes (e.g. 1, 2, 3, 4, 5, 6, or 7 minutes) after initiation of the color developing reaction in order to optimize signal to background ratio.

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In a particular preferred embodiment, the inventive antibody molecule comprises at least one CDR3 of an V<sub>1</sub>-region as encoded by a nucleic acid molecule as shown in SEQ ID NOs: 15, 17 or 19 or at least one CDR3 amino acid sequence of an V<sub>L</sub>region as shown in SEQ ID NOs: 16, 18 or 20 and/or said antibody molecule comprises at least one CDR3 of an V<sub>H</sub>-region as encoded by a nucleic acid molecule as shown in SEQ ID NOs: 21, 23 or 25 or at least one CDR3 amino acid sequence of an V<sub>H</sub>-region as shown in SEQ ID NOs: 22, 24 or 26. Most preferred are antibodies comprising at least one CDR3 of an V<sub>H</sub>-region as defined herein. The CDR-3 domains mentioned herein above relate to the inventive, illustrative parental antibody molecules MSR-3, -7, or -8. However, as illustrated in the appended tables 1, 8 or 10, matured and/or optimized antibody molecules obtainable by the methods disclosed in the appended examples may comprise modified V<sub>H</sub>-, V<sub>L</sub>-, CDR1, CDR2 and CDR3 regions. Accordingly, the antibody molecule of the invention is preferably selected from the group consisting of MSR-3, -7 and -8 or an affinity-matured version of MSR-3, -7 or -8. Affinity-matured as well as cross-cloned versions of MSR-3, -7 and -8 comprise, inter alia, antibody molecules comprising CDR1, CDR2 and/or CDR3 regions as shown in table 1 or 8 or characterized in any of SEQ ID NOs: 15 to 20, 21 to 26, 60 to 74, 75 to 87, 92 and 93 or 94 and 95 as well as in SEQ ID NOs: 354 to 413. Most preferably, the antibody of the invention comprises at least one CDR, preferably a CDR1, more preferably a CDR2, most preferably a CDR3 as shown in the appended table 1, 8 or as documented in appended table 10.

It is of note that affinity-maturation techniques are known in the art, described in the appended examples and, inter alia, in Knappik (2000), J. Mol. Biol. 296, 55; Krebs

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(2000), J. Imm. Meth. 254, 67-84; WO 01/87337; WO 01/87338; US 6,300,064; EP 96 92 92 78.8 and further references cited herein below.

In a more preferred embodiment of the invention, the antibody molecule is a full antibody (immunoglobulin, like an IgG1, an IgG2, an IgG2b, an IgG3, an IgG4, an IgA, an IgM, an IgD or an IgE), an F(ab)-, Fabc-, Fv-, Fab'-, F(ab')2- fragment, a single-chain antibody, a chimeric antibody, a CDR-grafted antibody, a bivalent antibody-construct, an antibody-fusion protein, a cross-cloned antibody or a synthetic antibody. Also envisaged are genetic variants of immunoglobulin genes. Genetic variants of, e.g., immunoglobulin heavy G chain subclass 1 (IgG1) may comprise the G1m(17) or G1m(3) allotypic markers in the CH1 domain, or the G1m(1) or the G1m(non-1) allotypic marker in the CH3 domain. The antibody molecule of the invention also comprises modified or mutant antibodies, like mutant IgG with enhanced or attenuated Fc-receptor binding or complement activation. It is also envisaged that the antibodies of the invention are produced by conventional means, e.g. the production of specific monoclonal antibodies generated by immunization of mammals, preferably mice, with peptides comprising the two regions of ßA4 as defined herein, e.g. the N-terminal and central region/epitope comprising (a) amino acids 2 to 10 (or (a) part(s) thereof) of  $\beta$ -A4 and (b) an amino acid stretch comprising amino acids 12 to 25 (or (a) part(s) thereof) of β-A4 (SEQ ID NO. 27). Accordingly, the person skilled in the art may generate monoclonal antibodies against such a peptide and may screen the obtained antibodies for the capacity to simultaneously and independently binding to/reacting with the N-terminal and central region/epitope of BA4 as defined herein. Corresponding screening methods are disclosed in the appended examples.

As illustrated in the appended examples, the inventive antibodies/antibody molecules can readily and preferably be recombinantly constructed and expressed. Preferably, the antibody molecule of the invention comprises at least one, more preferably at least two, preferably at least three, more preferably at least four, more preferably at least five and most preferably six CDRs of the herein defined MSR-3, MSR-7 or MSR-8 parental antibodies or of affinity-matured/optimized antibodies derived from said parental antibodies. It is of note that also more than six CDRs may

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be comprised in recombinantly produced antibodies of the invention. The person skilled in the art can readily employ the information given in the appended examples to deduce corresponding CDRs of the parental as well as the affinity optimized antibodies. Examples of optimized antibodies which have been obtained by maturation/optimization of the parental antibodies are, inter alia, shown in appended table 1. An maturated/optimized antibody molecule of the invention is, e.g. MSR 7.9H7 which is also characterized by sequences appended herein, which comprise SEQ ID NOs: 88 to 95 and which depict the V<sub>H</sub>-region of MSR 7.9H7 (SEQ ID NOs: 88 and 89), the V<sub>L</sub>-region of MSR 7.9H7 (SEQ ID NOs: 90 and 91), the H-CDR3 of MSR 7.9H7 (SEQ ID NOs: 92 and 93) as well as the L-CDR3 of MSR 7.9H7 (SEQ ID NOs: 94 and 95). Illustrative antibody molecule 7.9H7 is derived from parental antibody MSR7 and is a particular preferred inventive example of an optimized/matured antibody molecule of the present invention. This antibody molecule may be further modified in accordance with this invention, for example in form of cross-cloning, see herein below and appended examples.

As documented in the appended examples, the antibodies of the invention also comprise cross-cloned antibodies, i.e. antibodies comprising different antibody regions (e.g. CDR-regions) from one or more parental or affinity-optimized antibody(ies) as described herein. These cross-cloned antibodies may be antibodies in several, different frameworks, whereby the most preferred framework is an IgG-framework, even more preferred in an IgG1-, IgG2a or an IgG2b-framework. It is particularly preferred that said antibody framework is a mammalian, most preferably a human framework. The domains on the light and heavy chains have the same general structure and each domain comprises four framework regions, whose sequences are relatively conserved, joined by three hypervariable domains known as complementarity determining regions (CDR1-3).

As used herein, a "human framework region" relates to a framework region that is substantially identical (about 85% or more, usually 90-95% or more) to the framework region of a naturally occurring human immunoglobulin. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDR's. The CDR's are primarily

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responsible for binding to an epitope of an antigen. It is of note that not only cross-cloned antibodies described herein may be presented in a preferred (human) antibody framework, but also antibody molecules comprising CDRs from, inter alia, the parental antibodies MSR-3, -7 or -8 as described herein or of matured antibodies derived from said parental antibodies, may be introduced in an immunoglobulin framework. Preferred frameworks are IgG1, IgG2a and IgG2b. Most preferred are human frameworks and human IgG1 frameworks.

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As shown in the appended examples, it is, inter alia possible, to transfer, by genetic engineering known in the art whole light chains from an optimized donor clone to an optimized recipient clone. Example for an optimized donor clone is, e.g. L-CDR1 (L1) and an example for an optimized recipient clone is H-CDR2 (H2). Epitope specificity may be conserved by combining clones which possess the same H-CDR-3 regions. Further details are given in illustrative Example 13.

Preferred cross-cloned antibody molecules of the invention are selected from the group consisting of MS-R #3.3H1x3.4L9, MS-R #3.4H1x3.4L9, MS-R #3.4H3x3.4L7, MS-R #3.4H3x3.4L9, MS-R #3.4H7x3.4L9, MS-R #3.4H7x3.4L7, MS-R #3.6H5x3.6L1, MS-R #3.6H5x3.6L2, MS-R #3.6.H8x3.6.L2, MS-R #7.2H2x7.2L1, MS-R #7.4H2x7.2L1, MS-R #7.4H2x7.12L2, MS-R #7.9H2x7.2L1(L1), MS-R #7.9H2x7.12L1, MS-R #7.9H2x7.12L2, MS-R #7.9H2x7.12L2(L1+2), MS-R #7.9H4x7.12.L2. MS-R #7.11H1x7.2L1, MS-R #7.11H1x7.11L1 MS-R #7.11H2x7.2L1(L1), MS-R #7.11H2x7.9L1 (L1), MS-R #7.11H2x7.12L1 or MS-R #8.1H1x8.2L1.

The generation of cross-cloned antibodies is also illustrated in the appended examples. The above mentioned preferred cross-cloned antibodies/antibody molecules are optimized/matured antibody molecules derived from parental antibodies disclosed herein, in particular from MSR-3 and MSR-7. in addition, further characterizing CDR-sequences and V-regions of the cross-cloned antibody molecules/antibodies are given in appended SEQ ID NOs: 32, 33, 46 and 47 (MSR 3.6H5x3.6.L2; V<sub>H</sub>-, V<sub>L</sub>-region); 34, 35, 48 and 49 (MSR 3.6H8x3.6.L2; V<sub>H</sub>-, V<sub>L</sub>-regions); 36, 37, 50 and 51 (MSR 7.4H2x7.2.L1; V<sub>H</sub>-, V<sub>L</sub>-regions); 38, 39, 52 and 53 (MSR 7.9H2x7.12.L2; V<sub>H</sub>-, V<sub>L</sub>-regions); 40, 41, 54 and 55 (MSR # 7.9H4x7.12.L2;

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V<sub>H</sub>-, V<sub>L</sub>-regions); 42, 43, 56 and 57 (MSR #7.11H1x7.11.L1; V<sub>H</sub>-, V<sub>L</sub>-regions); and 44, 45, 58 and 59 (MSR # 7.11H1x7.2.L1; V<sub>H</sub>-, V<sub>L</sub>-regions). Corresponding CDR3 regions of these particular preferred cross-cloned antibody molecules are depicted in SEQ ID NOs: 60 to 87. For further MSR antibody molecules, V<sub>H</sub>-, V<sub>L</sub>-, CDR-regions can be deduced from appended Tables 8 or 10 and from the appended sequence listing, in particular SEQ ID NOS: 32 to 95 for MS-R antibodies/antibody molecules  $\#3.6H5 \times 3.6L2, \#3.6H8 \times 3.6L2, \#7.4H2 \times 7.2L1, \#7.9H2 \times 7.12L2, \#7.9H4 \times 7.12L2,$ #7.11H1 x 7.11L1, #7.11H1 x 7.2L1 and #7.9H7 or SEQ ID NOS: 294 to 413 for MSR-R antibodies/antibody molecules MS-R #3.3H1x3.4L9, #3.4H1 x 3.4L9, #3.4H3 x 3.4L7, #3.4H3 x 3.4L9, #3.4H7 x 3.4L9, #3.4H7 x 3.4L7, #3.6H5 x 3.6L1, #7.2H2 x 7.2L1,  $\#7.4H2 \times 7.12L2$ ,  $\#7.9H2 \times 7.2L1$ ,  $\#7.9H2 \times 7.12L1$ ,  $\#7.11H2 \times 7.2L1$ ,  $\#7.11H2 \times 7.9L1$ ,  $\#7.11H2 \times 7.12L1$  or  $\#8.1H1 \times 8.2L1$ . Accordingly, besides  $V_{H^-}$ regions defined above, preferred antibody molecules of the invention may comprise V<sub>H</sub>-regions as defined in any one of SEQ ID NOs: 294 to 323. Similarly, SEQ ID NOs: 324 to 353 depict preferred V<sub>L</sub>-regions which, besides to V<sub>L</sub>-regions defined above which may be comprised in the inventive antibody molecules. Corresponding CDR-3 regions are defined above, as well as in additional sequences shown in SEQ ID NOs: 354 to 413.

Inventive antibody molecules can easily be produced in sufficient quantities, inter alia, by recombinant methods known in the art, see, e.g. Bentley, Hybridoma 17 (1998), 559-567; Racher, Appl. Microbiol. Biotechnol. 40 (1994), 851-856; Samuelsson, Eur. J. Immunol. 26 (1996), 3029-3034.

Theoretically, in soluble  $\beta$ -A4 (monomeric/oligomeric) both the N-terminal and the middle epitopes are accessible for antibody interaction and antibody molecules of the present invention may either bind to the N-terminal or middle epitope separately, but under these conditions maximum affinity will not be obtained. However, it is more likely that an optimal contact to the antibody paratope will be attained by simultaneous binding to both epitopes, i.e. similar to the interaction with aggregated  $\beta$ -A4. Thus, antibodies of the present invention are unique anti-A $\beta$  antibodies in that they bind to aggregated  $\beta$ -A4 (via interaction with the N-terminal and middle epitope), and at the same time are also able to stabilize and neutralize the

conformational epitope in soluble  $\beta$ -A4. These antibodies are distinct to prior art antibodies.

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Most preferred are antibody molecules of the invention which have an affinity to  $A\beta$  or defined fragments thereof with a  $K_D$  value lower than 2000 nM, preferably lower than 100 nM, more preferably lower than 10 nM, most preferably lower than 1 nM. The measurement of such affinity/affinities may be carried out by methods illustrated in the examples and known in the art. Such methods comprise, but are not limited to BIACORE<sup>TM</sup>-assays (www.biacore.com; Malmquist (1999), Biochem.Soc. Trans 27, 335-340) and solid phase assays using labeled antibodies or labeled  $A\beta$ .

Preferably, the antibody molecule of the invention is capable of decorating/reacting with/binding to amyloid plaques in in vitro (post-mortem) brain sections from patients suffering from amyloid-related disorders, like Alzheimer's disease. Yet, it is also preferred that the inventive antibody/antibody molecules prevent A $\beta$ -aggregation in vivo as well as in in vitro assays, as illustrated in the appended examples. Similarly, the antibody molecules of the present invention are preferred to de-polymerize A $\beta$ -aggregate in vivo and/or in in vitro assays shown in the examples. This capacity of the inventive antibodies/antibody molecules is, inter alia, to be employed in medical settings, in particular in pharmaceutical compositions described herein below.

The invention also provides for a nucleic acid molecule encoding an inventive antibody molecule as defined herein.

Said nucleic acid molecule may be a naturally nucleic acid molecule as well as a recombinant nucleic acid molecule. The nucleic acid molecule of the invention may, therefore, be of natural origin, synthetic or semi-synthetic. It may comprise DNA, RNA as well as PNA and it may be a hybrid thereof.

It is evident to the person skilled in the art that regulatory sequences may be added to the nucleic acid molecule of the invention. For example, promoters, transcriptional enhancers and/or sequences which allow for induced expression of the polynucleotide of the invention may be employed. A suitable inducible system is for

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example tetracycline-regulated gene expression as described, e.g., by Gossen and Bujard (Proc. Natl. Acad. Sci. USA 89 (1992), 5547-5551) and Gossen et al. (Trends Biotech. 12 (1994), 58-62), or a dexamethasone-inducible gene expression system as described, e.g. by Crook (1989) EMBO J. 8, 513-519.

Furthermore, it is envisaged for further purposes that nucleic acid molecule may contain, for example, thioester bonds and/or nucleotide analogues. Said modifications may be useful for the stabilization of the nucleic acid molecule against endo- and/or exonucleases in the cell. Said nucleic acid molecules may be transcribed by an appropriate vector containing a chimeric gene which allows for the transcription of said nucleic acid molecule in the cell. In this respect, it is also to be understood that the polynucleotide of the invention can be used for "gene targeting" or "gene therapeutic" approaches. In another embodiment said nucleic acid molecules are labeled. Methods for the detection of nucleic acids are well known in the art, e.g., Southern and Northern blotting, PCR or primer extension. This embodiment may be useful for screening methods for verifying successful introduction of the inventive nucleic acid molecules during gene therapy approaches.

The nucleic acid molecule(s) of the invention may be a recombinantly produced chimeric nucleic acid molecule comprising any of the aforementioned nucleic acid molecules either alone or in combination. Preferably, the nucleic acid molecule of the invention is part of a vector.

The present invention therefore also relates to a vector comprising the nucleic acid molecule of the present invention.

The vector of the present invention may be, e.g., a plasmid, cosmid, virus, bacteriophage or another vector used e.g. conventionally in genetic engineering, and may comprise further genes such as marker genes which allow for the selection of said vector in a suitable host cell and under suitable conditions.

Furthermore, the vector of the present invention may, in addition to the nucleic acid sequences of the invention, comprise expression control elements, allowing proper expression of the coding regions in suitable hosts. Such control elements are known to the artisan and may include a promoter, a splice cassette, translation initiation

codon, translation and insertion site for introducing an insert into the vector. Preferably, the nucleic acid molecule of the invention is operatively linked to said expression control sequences allowing expression in eukaryotic or prokaryotic cells.

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Control elements ensuring expression in eukaryotic and prokaryotic cells are well known to those skilled in the art. As mentioned herein above, they usually comprise regulatory sequences ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript. Additional regulatory elements may include transcriptional as well as translational enhancers, and/or naturally-associated or heterologous promoter regions. Possible regulatory elements permitting expression in for example mammalian host cells comprise the CMV- HSV thymidine kinase promoter, SV40, RSV-promoter (Rous Sarcoma Virus), human elongation factor 1α-promoter, the glucocorticoid-inducible MMTV-promoter (Moloney Mouse Tumor Virus), metallothionein- or tetracyclin-inducible promoters, or enhancers, like CMV enhancer or SV40-enhancer. For expression in neural cells, it is envisaged that neurofilament-, PGDF-, NSE-, PrP-, or thy-1-promoters can be employed. Said promoters are known in the art and, inter alia, described in Charron (1995), J. Biol. Chem. 270, 25739-25745. For the expression in prokaryotic cells, a multitude of promoters including, for example, the tac-lac-promoter or the trp promoter, has been described. Besides elements which are responsible for the initiation of transcription such regulatory elements may also comprise transcription termination signals, such as SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. In this context, suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pRc/CMV, pcDNA1, pcDNA3 (In-vitrogene), pSPORT1 (GIBCO BRL), pX (Pagano (1992) Science 255, 1144-1147), yeast two-hybrid vectors, such as pEG202 and dpJG4-5 (Gyuris (1995) Cell 75, 791-803), or prokaryotic expression vectors, such as lambda gt11 or pGEX (Amersham-Pharmacia). Beside the nucleic acid molecules of the present invention, the vector may further comprise nucleic acid sequences encoding for secretion signals. Such sequences are well known to the person skilled in the art. Furthermore, depending on the expression system used leader sequences capable of directing the peptides of the invention to a cellular compartment may be added to the coding sequence of the nucleic acid molecules of the invention and are well

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known in the art. The leader sequence(s) is (are) assembled in appropriate phase with translation, initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein, or a protein thereof, into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an C- or N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and, as desired, the collection and purification of the antibody molecules or fragments thereof of the invention may follow. The invention also relates, accordingly, to hosts/host cells which comprise a vector as defined herein. Such hosts may be useful for in processes for obtaining antibodies/antibody molecules of the invention as well as in medical/pharmaceutical settings. Said host cells may also comprise transduced or transfected neuronal cells, like neuronal stem cells, preferably adult neuronal stem cells. Such host cells may be useful in transplantation therapies.

Furthermore, the vector of the present invention may also be an expression, a gene transfer or gene targeting vector. Gene therapy, which is based on introducing therapeutic genes into cells by ex-vivo or in-vivo techniques is one of the most important applications of gene transfer. Transgenic mice expressing a neutralizing antibody directed against nerve growth factor have been generated using the "neuroantibody" technique; Capsoni, Proc. Natl. Acad. Sci. USA 97 (2000), 6826-6831 and Biocca, Embo J. 9 (1990), 101-108. Suitable vectors, methods or genedelivering systems for in-vitro or in-vivo gene therapy are described in the literature and are known to the person skilled in the art; see, e.g., Giordano, Nature Medicine 2 (1996), 534-539; Schaper, Circ. Res. 79 (1996), 911-919; Anderson, Science 256 (1992), 808-813, Isner, Lancet 348 (1996), 370-374; Muhlhauser, Circ. Res. 77 (1995), 1077-1086; Onodua, Blood 91 (1998), 30-36; Verzeletti, Hum. Gene Ther. 9 (1998), 2243-2251; Verma, Nature 389 (1997), 239-242; Anderson, Nature 392 (Supp. 1998), 25-30; Wang, Gene Therapy 4 (1997), 393-400; Wang, Nature Medicine 2 (1996), 714-716; WO 94/29469; WO 97/00957; US 5,580,859; US 5,589,466; US 4,394,448 or Schaper, Current Opinion in Biotechnology 7 (1996),

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635-640, and references cited therein. In particular, said vectors and/or gene delivery systems are also described in gene therapy approaches in neurological tissue/cells (see, inter alia Blömer, J. Virology 71 (1997) 6641-6649) or in the hypothalamus (see, inter alia, Geddes, Front Neuroendocrinol. 20 (1999), 296-316 or Geddes, Nat. Med. 3 (1997), 1402-1404). Further suitable gene therapy constructs for use in neurological cells/tissues are known in the art, for example in Meier (1999), J. Neuropathol. Exp. Neurol. 58, 1099-1110. The nucleic acid molecules and vectors of the invention may be designed for direct introduction or for introduction via liposomes, viral vectors (e.g. adenoviral, retroviral), electroporation, ballistic (e.g. gene gun) or other delivery systems into the cell. Additionally, a baculoviral system can be used as eukaryotic expression system for the nucleic acid molecules of the invention. The introduction and gene therapeutic approach should, preferably, lead to the expression of a functional antibody molecule of the invention, whereby said expressed antibody molecule is particularly useful in the treatment, amelioration and/or prevention of neurological disorders related to abnormal amyloid synthesis, assembly and/or aggregation, like, Alzheimer's disease and the like.

Accordingly, the nucleic acid molecule of the present invention and/or the above described vectors/hosts of the present invention may be particularly useful as pharmaceutical compositions. Said pharmaceutical compositions may be employed in gene therapy approaches. In this context, it is envisaged that the nucleic acid molecules and/or vectors of the present invention may be employed to modulate, alter and/or modify the (cellular) expression and/or concentration of the antibody molecules of the invention or of (a) fragment(s) thereof.

For gene therapy applications, nucleic acids encoding the peptide(s) of the invention or fragments thereof may be cloned into a gene delivering system, such as a virus and the virus used for infection and conferring disease ameliorating or curing effects in the infected cells or organism.

The present invention also relates to a host cell transfected or transformed with the vector of the invention or a non-human host carrying the vector of the present invention, i.e. to a host cell or host which is genetically modified with a nucleic acid

molecule according to the invention or with a vector comprising such a nucleic acid molecule. The term "genetically modified" means that the host cell or host comprises in addition to its natural genome a nucleic acid molecule or vector according to the invention which was introduced into the cell or host or into one of its predecessors/parents. The nucleic acid molecule or vector may be present in the genetically modified host cell or host either as an independent molecule outside the genome, preferably as a molecule which is capable of replication, or it may be stably integrated into the genome of the host cell or host.

The host cell of the present invention may be any prokaryotic or eukaryotic cell. Suitable prokaryotic cells are those generally used for cloning like E. coli or Bacillus subtilis. Furthermore, eukaryotic cells comprise, for example, fungal or animal cells. Examples for suitable fungal cells are yeast cells, preferably those of the genus Saccharomyces and most preferably those of the species Saccharomyces cerevisiae. Suitable animal cells are, for instance, insect cells, vertebrate cells, preferably mammalian cells, such as e.g. HEK293, NSO, CHO, MDCK, U2-OSHela, NIH3T3, MOLT-4, Jurkat, PC-12, PC-3, IMR, NT2N, Sk-n-sh, CaSki, C33A. These host cells, e.g. CHO-cells, may provide post-translational modifications to the antibody molecules of the invention, including leader peptide removal, folding and assembly of H (heavy) and L (light) chains, glycosylation of the molecule at correct sides and secretion of the functional molecule. Further suitable cell lines known in the art are obtainable from cell line depositories, like the American Type Culture Collection (ATCC). In accordance with the present invention, it is furthermore envisaged that primary cells/cell cultures may function as host cells. Said cells are in particular derived from insects (like insects of the species Drosophila or Blatta) or mammals (like human, swine, mouse or rat). Said host cells may also comprise cells from and/or derived from cell lines like neuroblastoma cell lines. The above mentioned primary cells are well known in the art and comprise, inter alia, primary astrocytes, (mixed) spinal cultures or hippocampal cultures.

In a more preferred embodiment the host cell which is transformed with the vector of the invention is a neuronal cell, a neuronal stem cell (e.g. an adult neuronal stem cell), a brain cell or a cell (line) derived therefrom. However, also a CHO-cell

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comprising the nucleic acid molecule of the present invetion may be particularly useful as host. Such cells may provide for correct secondary modifications on the expressed molecules, i.e. the antibody molecules of the present invention. These modifications comprise, inter alia, glycosylations and phosphorylations.

Hosts may be non-human mammals, most preferably mice, rats, sheep, calves, dogs, monkeys or apes. Said mammals may be indispensable for developing a cure, preferably a cure for neurological and/or neurodegenerative disorders mentioned herein. Furthermore, the hosts of the present invention may be particularly useful in producing the antibody molecules (or fragments thereof) of the invention. It is envisaged that said antibody molecules (or fragments thereof) be isolated from said host. It is, inter alia, envisaged that the nucleic acid molecules and or vectors described herein are incorporated in sequences for transgenic expression. The introduction of the inventive nucleic acid molecules as transgenes into non-human hosts and their subsequent expression may be employed for the production of the inventive antibodies. For example, the expression of such (a) transgene(s) in the milk of the transgenic animal provide for means of obtaining the inventive antibody molecules in quantitative amounts; see inter alia, US 5,741,957, US 5,304,489 or US 5,849,992. Useful transgenes in this respect comprise the nucleic acid molecules of the invention, for example, coding sequences for the light and heavy chains of the antibody molecules described herein, operatively linked to promotor and/or enhancer structures from a mammary gland specific gene, like casein or beta-lactoglobulin.

The invention also provides for a method for the preparation of an antibody molecule of the invention comprising culturing the host cell described herein above under conditions that allow synthesis of said antibody molecule and recovering said antibody molecule from said culture.

The invention also relates to a composition comprising an antibody molecule of the invention or produced by the method described herein above, a nucleic acid molecule encoding the antibody molecule of the invention, a vector comprising said nucleic acid molecule or a host-cell as defined herein above and optionally, further molecules, either alone or in combination, like e.g. molecules which are capable of

interfering with the formation of amyloid plaques or which are capable of depolymerizing already formed amyloid-plaques. The term "composition" as employed herein comprises at least one compound of the invention. Preferably, such a composition is a pharmaceutical or a diagnostic composition.

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The composition may be in solid or liquid form and may be, inter alia, in a form of (a) powder(s), (a) tablet(s), (a) solution(s) or (an) aerosol(s). Said composition may comprise on or more antibodies/antibody molecules of the invention or nucleic acid molecules, vector or hosts of the invention. It is also envisaged that said composition comprises at least two, preferably three, more preferably four, most preferably five antibody molecules of the invention or nucleic acid molecule(s) encoding said antibody molecule(s). Said composition may also comprise optimized, inventive antibodies/antibody molecules obtainable by the methods described herein below and in the appended examples.

It is preferred that said pharmaceutical composition, optionally comprises a pharmaceutically acceptable carrier and/or diluent. The herein disclosed pharmaceutical composition may be particularly useful for the treatment of neurological and/or neurodegenerative disorders. Said disorders comprise, but are not limited to Alzheimer's disease, amyothrophic lateral sclerosis (ALS), hereditary cerebral hemorrhage with amyloidosis Dutch type, Down's syndrome, HIV-dementia, Parkinson's disease and neuronal disorders related to aging. The pharmaceutical composition of the invention is, inter alia, envisaged as potent inhibitors of amyloid plaque formation or as a potent stimulator for the de-polymerization of amyloid plaques. Therefore, the present invention provides for pharmaceutical compositions comprising the compounds of the invention to be used for the treatment of diseases/disorders associated with pathological APP proteolysis and/or amyloid plaque formation.

Examples of suitable pharmaceutical carriers, excipients and/or diluents are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Compositions comprising such carriers can be formulated by well known

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conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g., by intravenous, intraperitoneal, subcutaneous, intramuscular, topical, intradermal, intranasal or intrabronchial administration. It is particularly preferred that said administration is carried out by injection and/or delivery, e.g., to a site in a brain artery or directly into brain tissue. The compositions of the invention may also be administered directly to the target site, e.g., by biolistic delivery to an external or internal target site, like the brain. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Proteinaceous pharmaceutically active matter may be present in amounts between 1 ng and 10 mg/kg body weight per dose; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. If the regimen is a continuous infusion, it should also be in the range of 1 µg to 10 mg units per kilogram of body weight per minute.

Progress can be monitored by periodic assessment. The compositions of the invention may be administered locally or systemically. It is of note that peripherally administered antibodies can enter the central nervous system, see, inter alia, Bard (2000), Nature Med. 6, 916-919. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Furthermore, the pharmaceutical composition of the invention may comprise further agents depending on the intended use of the pharmaceutical composition. Said agents may be drugs acting on the

central nervous system, like, neuroprotective factors, cholinesterase inhibitors, agonists of M1 muscarinic receptor, hormones, antioxidants, inhibitors of inflammation etc. It is particularly preferred that said pharmaceutical composition comprises further agents like, e.g. neurotransmitters and/or substitution molecules for neurotransmitters, vitamin E, or alpha-lipoic acid.

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The pharmaceutical compositions, as well as the methods of the invention or the uses of the invention described infra can be used for the treatment of all kinds of diseases hitherto unknown or being related to or dependent on pathological APP aggregation or pathological APP processing. They may be particularly useful for the treatment of Alzheimer's disease and other diseases where extracellular deposits of amyloid- $\beta$ , appear to play a role. They may be desirably employed in humans, although animal treatment is also encompassed by the methods, uses and compositions described herein.

In a preferred embodiment of the invention, the composition of the present invention as disclosed herein above is a diagnostic composition further comprising, optionally, suitable means for detection. The diagnostic composition comprises at least one of the aforementioned compounds of the invention.

Said diagnostic composition may comprise the compounds of the invention, in particular and preferably the antibody molecules of the present invention, in soluble form/liquid phase but it is also envisaged that said compounds are bound to/attached to and/or linked to a solid support.

Solid supports may be used in combination with the diagnostic composition as defined herein or the compounds of the present invention may be directly bound to said solid supports. Such supports are well known in the art and comprise, inter alia, commercially available column materials, polystyrene beads, latex beads, magnetic beads, colloid metal particles, glass and/or silicon chips and surfaces, nitrocellulose strips, membranes, sheets, duracytes, wells and walls of reaction trays, plastic tubes etc. The compound(s) of the invention, in particular the antibodies of the present invention, may be bound to many different carriers. Examples of well-known carriers include glass, polystyrene, polyvinyl chloride, polypropylene, polyethylene,

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polycarbonate, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble or insoluble for the purposes of the invention. Appropriate labels and methods for labeling have been identified above and are furthermore mentioned herein below. Suitable methods for fixing/immobilizing said compound(s) of the invention are well known and include, but are not limited to ionic, hydrophobic, covalent interactions and the like.

It is particularly preferred that the diagnostic composition of the invention is employed for the detection and/or quantification of APP and/or APP-processing products, like amyloid- $\beta$  or for the detection and/or quantification of pathological and/or (genetically) modified APP-cleavage sides.

As illustrated in the appended examples, the compounds of the present invention, in particular the inventive antibody molecules are particularly useful as diagnostic reagents in the detection of genuine human amyloid plaques in brain sections of Alzheimer's Disease patients by indirect immunofluorescence.

It is preferred that said compounds of the present invention to be employed in a diagnostic composition are detectably labeled. A variety of techniques are available for labeling biomolecules, are well known to the person skilled in the art and are considered to be within the scope of the present invention. Such techniques are, e.g., described in Tijssen, "Practice and theory of enzyme immuno assays", Burden, RH and von Knippenburg (Eds), Volume 15 (1985), "Basic methods in molecular biology"; Davis LG, Dibmer MD; Battey Elsevier (1990), Mayer et al., (Eds) "Immunochemical methods in cell and molecular biology" Academic Press, London (1987), or in the series "Methods in Enzymology", Academic Press, Inc.

There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, colloidal metals, fluorescent compounds, chemiluminescent compounds, and bioluminescent compounds.

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Commonly used labels comprise, inter alia, fluorochromes (like fluorescein, rhodamine, Texas Red, etc.), enzymes (like horse radish peroxidase,  $\beta$ -galactosidase, alkaline phosphatase), radioactive isotopes (like  $^{32}P$  or  $^{125}I$ ), biotin, digoxygenin, colloidal metals, chemi- or bioluminescent compounds (like dioxetanes, luminol or acridiniums). Labeling procedures, like covalent coupling of enzymes or biotinyl groups, iodinations, phosphorylations, biotinylations, etc. are well known in the art.

Detection methods comprise, but are not limited to, autoradiography, fluorescence microscopy, direct and indirect enzymatic reactions, etc. Commonly used detection assays comprise radioisotopic or non-radioisotopic methods. These comprise, inter alia, Westernblotting, overlay-assays, RIA (Radioimmuno Assay) and IRMA (Immune Radioimmunometric Assay), EIA (Enzyme Immuno Assay), ELISA (Enzyme Linked Immuno Sorbent Assay), FIA (Fluorescent Immuno Assay), and CLIA (Chemioluminescent Immune Assay).

Furthermore, the present invention provides for the use of an antibody molecule of invention, or an antibody molecule produced by the method of the invention, of a nucleic acid molecule, vector of or a host of the invention for the preparation of a pharmaceutical or a diagnostic composition for the prevention, treatment and/or diagnosis of a disease associated with amyloidogenesis and/or amyloid-plaque formation. It is further preferred that the compounds described herein, in particular the antibody molecules of the invention, be employed in the prevention and/or treatment of neuropathologies associated with modified or abnormal APP-processing and/or amyloidogenesis. The antibody molecules, e.g in format of (engineered) immunoglobulins, like antibodies in a IgG framework, in particular in an IgG1framework, or in the format of chimeric antibodies, bispecific antibodies, single chain Fvs (scFvs) or bispecific scFvs and the like are to employed in the preparation of the pharmaceutical compositions provided herein. Yet, the antibody molecules are also useful in diagnostic settings as documented in the appended examples, since the antibody molecules of the invention specifically interact with/detect Aß4 and/or amyloid deposits/plaques.

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Therefore an inventive use of the compounds of the present invention is the use for the preparation of a pharmaceutical composition for a neurological disorder which calls for amelioration, for example by disintegration of  $\beta$ -amyloid plaques, by amyloid (plague) clearance or by passive immunization against  $\beta$ -amyloid plague formation. As illustrated in the appended examples, the inventive antibody molecules are particularly useful in preventing AB aggregation and in de-polymerization of already formed amyloid aggregates. Accordingly, the inventive antibodies are to be employed in the reduction of pathological amyloid deposits/plagues, in the clearance of amyloid plaques/plaque precursors as well as in neuronal protection. It is in particular envisaged that the antibody molecules of the invention be employed in the in vivo prevention of amyloid plaques as well as in in vivo clearance of pre-existing amyloid plaques/deposits. Furthermore, the antibody molecules of the invention may be employed in passive immunization approaches against Aß4. Clearance of Aß4/Aß4 deposits may, inter alia, be achieved by the medical use of antibodies of the present invention which comprise an Fc-part. Said Fc-part of an antibody may be particularly useful in Fc-receptor mediated immune responses, e.g. the attraction of macrophages (phagocytic cells and/or microglia) and/or helper cells. For the mediation of Fc-part-related immunoresponses, the antibody molecule of the invention is preferably in an (human) IgG1- framework. As discussed herein, the preferred subject to be treated with the inventive antibody molecules, the nucleic acid molecules encoding the same or parts thereof, the vectors of the invention or the host cells of this invention is a human subject. Other frameworks, like IgG2a- or IgG2b-frameworks for the inventive antibody molecules are also envisaged. Immunoglobulin frameworks in IgG2a und IgG2b format are particular envisaged in mouse settings, for example in scientific uses of the inventive antibody molecules, e.g. in tests on transgenic mice expressing (human) wildtype or mutated APP, APPfragments and/or Aß4.

The above recited diseases associated with amyloidogenesis and/or amyloid-plaque formation comprise, but are not limited to dementia, Alzheimer's disease, motor neuropathy, Parkinson's disease, ALS (amyotrophic lateral sclerosis), scrapie, HIV-related dementia as well as Creutzfeld-Jakob disease, hereditary cerebral hemorrhage, with amyloidis Dutch type, Down's syndrome and neuronal disorders

related to aging. The antibody molecules of the invention and the compositions provided herein may also be useful in the amelioration and or prevention of inflammatory processes relating to amyloidogenesis and/or amyloid plaque formation.

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Accordingly, the present invention also provides for a method for treating, preventing and/or delaying neurological and/or neurodegenerative disorders comprising the step of administering to a subject suffering from said neurological and/or neurodegenerative disorder and/or to a subject susceptible to said neurological and/or neurodegenerative disorder an effective amount of a antibody molecule of the invention, a nucleic acid molecule of invention and/or a composition as defined herein above.

In yet another embodiment, the present invention provides for a kit comprising at least one antibody molecule, at least one nucleic acid molecule, at least one vector or at least one host cell of the invention. Advantageously, the kit of the present invention further comprises, optionally (a) buffer(s), storage solutions and/or remaining reagents or materials required for the conduct of medical, scientific or diagnostic assays and purposes. Furthermore, parts of the kit of the invention can be packaged individually in vials or bottles or in combination in containers or multicontainer units.

The kit of the present invention may be advantageously used, inter alia, for carrying out the method of the invention and could be employed in a variety of applications referred herein, e.g., as diagnostic kits, as research tools or medical tools. Additionally, the kit of the invention may contain means for detection suitable for scientific, medical and/or diagnostic purposes. The manufacture of the kits follows preferably standard procedures which are known to the person skilled in the art.

The invention also provides for a method for the optimization of an antibody molecule as defined herein above comprising the steps of

(a) constructing a library of diversified Fab antibody fragments derived from an antibody comprising at least one CDR3 of an V<sub>H</sub>-region as encoded by a nucleic acid molecule as

shown in SEQ ID NOs: 21, 23 or 25 or at least one CDR3 amino acid sequence of an  $V_{H}$ -region as shown in SEQ ID NOs: 22, 24 or 26;

- (b) testing the resulting Fab optimization library by panning against A $\beta$ /A $\beta$ 4;
- (c) identifying optimized clones; and
- (d) expressing of selected, optimized clones.

Optimization of the antibodies/antibody molecules of the invention is also documented in the appended examples and may comprise the selection for, e.g. higher affinity for one or both regions/epitopes of  $\beta$ -A4 as defined herein or selection for improved expression and the like. In one embodiment, said selection for to higher affinity for one or both regions/epitopes of  $\beta$ -A4 comprises the selection for high affinity to (a) an amino acid stretch comprising amino acids 2 to 10 (or (a) part(s) thereof) of  $\beta$ -A4 and/or (b) an amino acid stretch comprising amino acids 12 to 25 (or (a) part(s) thereof) of  $\beta$ -A4 (SEQ ID NO. 27).

The person skilled in the art can readily carry out the inventive method employing the teachings of the present invention. Optimization protocols for antibodies are known in the art. These optimization protocols comprise, inter alia, CDR walking mutagenesis as disclosed and illustrated herein and described in Yang (1995), J. Mol. Biol. 25, 392-403; Schier (1996), J. Mol. Biol. 263, 551-567; Barbas (1996), Trends. Biotech 14, 230-34 or Wu (1998), PNAS 95, 6037-6042; Schier (1996), Human Antibodies Hybridomas 7, 97; Moore (1997), J. Mol. Biol. 272, 336.

"Panning"-techniques are also known in the art, see, e.g. Kay (1993), Gene 128, 59-65. Furthermore, publications like Borrebaeck (1995), "Antibody Engineering", Oxford University, 229-266; McCafferty (1996), "Antibody Engineering", Oxford University Press; Kay (1996), A Laboratory Manual, Academic Press provide for optimization protocols which may be modified in accordance with this invention.

The optimization method may further comprise a step (ca), whereby the optimized clones are further optimized by cassette mutagenesis, as illustrated in the appended examples.

The method for the optimization of an antibody molecule described herein is further illustrated in the appended examples as affinity maturation of parental antibodies

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/antibody molecules capable of specifically recognizing two regions of the beta -A4 peptide/ Abeta4/ Aβ/Aβ4/βA4.

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Preferably, said  $A\beta/A\beta4$  (also designated as &A4 in context of this invention) in step (b) of the method described herein above is aggregated  $A\beta/A\beta4$ . Said panning may be carried out (as described in the appended examples) with increased stringency of binding. Stringency may be increased, inter alia, by reducing the  $A\beta/A\beta4$  concentration or by elevating the (assay) temperature. The testing of the optimized library by panning is known to the skilled artisan and described in Kay (1993), loc. cit. Preferably, the identification in step (c) is carried out by ranking according to the lowest  $K_D$ -values.

Most preferably said identification in step (c) is carried out by koff-ranking. Koff-ranking is known to the skilled artisan and described in Schier (1996), loc. cit.; Schier (1996), J. Mol. Biol. 255, 28-43 or Duenas (1996), Mol. Immunol. 33, 279-286. Furthermore, koff-ranking is illustrated in the appended examples. The off-rate constant may be measured as described in the appended examples.

As mentioned herein above, the identified clones may, for further evaluation, be expressed. The expression may be carried out by known methods, inter alia, illustrated in the appended examples. The expression may, inter alia, lead to expressed Fabfragments, scFvs, bispecific immunoglobulins, bispecific antibody molecules, Fab- and/or Fv fusion proteins, or full antibodies, like IgGs, in particular IgG1.

Optimized antibodies, in particular optimized Fabs or optimized IgGs, preferably IgG1s, may be tested by methods as illustrated in the appended examples. Such methods comprise, but are not limited to, the testing of binding affinities, the determination of  $K_D$  values, pepspot analysis, ELISA-assays, RIA-assays, CLIA-assays, (immuno-) histological studies (for example staining of amyloid plaques), depolymerization assays or antibody-dependent  $\beta$ -A4 phagocytoses.

In a further embodiment of the present invention, a method is provided wherein optimized antibodies are generated by cross-cloning. This method is also illustrated in the appended examples and comprises the step of combining independently

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optimized CDR-regions, for example, by combining independently optimized H-CDR2 and L-CDR2 from matured clones with H-CDR3, preferably the same H-CDR3.

In a preferred embodiment, the invention relates to a method for the preparation of a pharmaceutical composition comprising the steps of

- (a) optimization of an antibody according to the method described herein and illustrated in the appended examples; and
- (b) formulating the optimized antibody/antibody molecule with an physiologically acceptable carrier, as described herein above.

Accordingly, the invention also provides for a pharmaceutical composition prepared by the method disclosed herein and comprising further optimized antibody molecules capable of specifically recognizing two regions of the beta-A4 petide/Abeta4/Aß/A4ß/BA4, as described herein above.

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# **Exemplified Sequences as recited herein:**

SEQ ID NO: 1

**AEFRHDSGY** 

First region of β-A4 peptide, "N-terminal region/epitope"

SEQ ID NO: 2

VHHQKLVFFAEDVG

Second region of β-A4 peptide, "Central/middle region/epitope"

SEQ ID NO: 3

VH-region of MS-Roche#3 (nucleic acid sequence)

SEQ ID NO: 4

VH-region of MS-Roche#3 (amino acid sequence)

QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGS GGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARLTHYARYYRYF DVWGQGTLVTVSS (SEQ ID NO : 4)

SEQ ID NO: 5

VH-region of MS-Roche#7 (nucleic acid sequence)

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SEQ ID NO: 6

VH-region of MS-Roche#7 (amino acid sequence)

QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGS GGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGKGNTHKPYGY VRYFDVWGQGTLVTVSS (SEQ ID NO: 6)

SEQ ID NO: 7

VH-region of MS-Roche#8 (nucleic acid sequence)

SEQ ID NO: 8

VH-region of MS-Roche#8 (amino acid sequence)
QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGS
GGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARLLSRGYNGYYH
KFDVWGQGTLVTVSS (SEQ ID NO: 8)

SEQ ID NO: 9

VL-region of MS-Roche#3 (nucleic acid sequence)
GATATCGTGCTGACCCAGAGCCCGGCGACCCTGAGCCTGTCTCCGGGCGAAC
GTGCGACCCTGAGCTGCAGAGCGAGCCAGAGCGTGAGCAGCAGCTATCTGGC
GTGGTACCAGCAGAAACCAGGTCAAGCACCGCGTCTATTAATTTATGGCGCGA
GCAGCCGTGCAACTGGGGTCCCGGCGCGTTTTAGCGGCTCTGGATCCGGCAC
GGATTTTACCCTGACCATTAGCAGCCTGGAACCTGAAGACTTTGCGGTTTATTA
TTGCCAGCAGGTTTATAATCCTCCTGTTACCTTTGGCCAGGGTACGAAAGTTGA
AATTAAACGTACG (SEQ ID NO: 9)

SEQ ID NO: 10

VL-region of MS-Roche #3 (amino acid sequence)
DIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA
TGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQVYNPPVTFGQGTKVEIKRT
(SEQ ID NO: 10)

SEQ ID NO: 11

VL-region of MS-Roche#7 (nucleic acid sequence)
GATATCGTGCTGACCCAGAGCCCGGCGACCCTGAGCCTGTCTCCGGGCGAAC
GTGCGACCCTGAGCTGCAGAGCGAGCCAGAGCGTGAGCAGCTATCTGGC

GTGGTACCAGCAGAAACCAGGTCAAGCACCGCGTCTATTAATTTATGGCGCGA GCAGCCGTGCAACTGGGGTCCCGGCGCGTTTTAGCGGCTCTGGATCCGGCAC GGATTTTACCCTGACCATTAGCAGCCTGGAACCTGAAGACTTTGCGACTTATTA TTGCTTTCAGCTTTATTCTGATCCTTTTACCTTTGGCCAGGGTACGAAAGTTGAA ATTAAACGTACG (SEQ ID NO. 11)

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SEQ ID NO: 12

VL-region of MS-Roche#7 (amino acid sequence)
DIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA
TGVPARFSGSGSGTDFTLTISSLEPEDFATYYCFQLYSDPFTFGQGTKVEIKRT
(SEQ ID NO: 12)

SEQ ID NO: 13

VL-region of MS-Roche#8 (nucleic acid sequence)
GATATCGTGCTGACCCAGAGCCCGGCGACCCTGAGCCTGTCTCCGGGCGAAC
GTGCGACCCTGAGCTGCAGAGCGAGCCAGAGCGTGAGCAGCAGCTATCTGGC
GTGGTACCAGCAGAAACCAGGTCAAGCACCGCGTCTATTAATTTATGGCGCGA
GCAGCCGTGCAACTGGGGTCCCGGCGCGTTTTAGCGGCTCTGGATCCGGCAC
GGATTTTACCCTGACCATTAGCAGCCTGGAACCTGAAGACTTTGCGACTTATTA
TTGCCAGCAGCTTTCTTTTTCCTCCTACCTTTGGCCAGGGTACGAAAGTTGA
AATTAAACGTACG (SEQ ID NO: 13)

SEQ ID NO: 14

VL-region of MS-Roche#8 (amino acid sequence)
DIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA
TGVPARFSGSGSGTDFTLTISSLEPEDFATYYCQQLSSFPPTFGQGTKVEIKRT
(SEQ ID NO: 14)

SEQ ID NO: 15

CDR3 of V<sub>1</sub>-region of MSR-3 (nucleic acid sequence)

CAGCAGGTTTATAATCCTCCTGTT

(SEQ ID NO: 15)

SEQ ID NO: 16

CDR3 of V<sub>L</sub>-region of MSR-3 (amino acid sequence)

QQVYNPPV (SEQ ID NO: 16)

SEQ ID NO: 17

CDR3 of V<sub>L</sub>-region of MSR-7 (nucleic acid sequence)

TTTCAGCTTTATTCTGATCCTTTT

(SEQ ID NO: 17)

SEQ ID NO: 18

CDR3 of V<sub>L</sub>-region of MSR-7 (amino acid sequence)

FQLYSDPF (SEQ ID NO. 18)

SEQ ID NO: 19

CDR3 of V<sub>L</sub>-region of MSR-8 (nucleic acid sequence)

CAGCAGCTTTCTTCTTTTCCTCCT

(SEQ ID NO. 19)

SEQ ID NO: 20

CDR3 of V<sub>L</sub>-region of MSR-8 (amino acid sequence)

QQLSSFPP (SEQ ID NO: 20)

SEQ ID NO: 21

CDR of V<sub>H</sub>-region of MSR-3 (nucleic acid sequence)

CTTACTCATTATGCTCGTTATTATCGTTATTTTGATGTT

(SEQ ID NO: 21)

SEQ ID NO: 22

CDR of V<sub>H</sub>-region of MSR-3 (amino acid sequence)

LTHYARYYRYFDV (SEQ ID NO: 22)

SEQ ID NO: 23

CDR of V<sub>H</sub>-region of MSR-7 (nucleic acid sequence)

GGTAAG GGTAATACT CATAAG CCT TAT GGTTAT GTT CGT TAT TTT GAT GTT

(SEQ ID NO: 23)

SEQ ID NO: 24

CDR of V<sub>H</sub>-region of MSR-7 (amino acid sequence)

GKGNTHKPYGYVRYFDV (SEQ ID NO: 24)

SEQ ID NO: 25

CDR of V<sub>H</sub>-region of MSR-8 (nucleic acid sequence)

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CTTCTTTCTCGTGGTTATAATGGTTATTATCATAAGTTTGATGTT (SEQ ID NO. 25)

SEQ ID NO: 26

CDR of V<sub>H</sub>-region of MSR-8 (amino acid sequence)

LLSRGYNGYYHKFDV (SEQ ID NO: 26)

SEQ ID NO: 27 Aβ4 (amino acids 1 to 42)

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA (SEQ ID NO: 27)

SEQ ID NO: 28 primer

5'-GTGGTGGTTCCGATATC-3' (SEQ ID NO: 28)

SEQ ID NO: 29 primer

5'- AGCGTCACACTCGGTGCGGCTTTCGGCTGGCCAAGAACGGTTA-3' (SEQ ID

NO: 29)

SEQ ID NO: 30 primer

5'-CAGGAAACAGCTATGAC-3' (SEQ ID NO: 30)

SEQ ID NO: 31 primer

5'-TACCGTTGCTCTTCACCCC-3' (SEQ ID NO: 31)

SEQ ID NO. 33: prot VH region of MS-Roche#3.6H5 x 3.6L2; protein/1; artificial sequence

QLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISESGK TKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARLTHYARYYRYFDV WGQGTLVTVSS (SEQ ID NO: 33)

SEQ ID NO: 35 prot VH region of MS-Roche#3.6H8 x 3.6L2; protein/1; artificial sequence

QLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISEYSK FKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARLTHYARYYRYFDV WGQGTLVTVSS (SEQ ID NO: 35)

SEQ ID NO: 37 prot VH region of MS-Roche#7.4H2 x 7.2L1; protein/1; artificial sequence

QLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAINYNGA RIYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGKGNTHKPYGYVRY FDVWGQGTLVTVSS (SEQ ID NO: 37)

SEQ ID NO: 39 prot VH region of MS-Roche#7.9H2 x 7.12 L2; protein/1; artificial sequence

QLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAINADGN RKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGKGNTHKPYGYVR YFDVWGQGTLVTVSS (SEQ ID NO: 39)

SEQ ID NO: 41 prot VH region of MS-Roche#7.9H4 x 7.12L2; protein/1; artificial sequence

QLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAINAVGM KKFYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGKGNTHKPYGYVR YFDVWGQGTLVTVSS (SEQ ID NO: 41)

SEQ ID NO. 43 prot VH region of MS-Roche#7.11H1 x 7.11L1; protein/1; artificial sequence

QLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSGINAAGF RTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGKGNTHKPYGYVR YFDVWGQGTLVTVSS (SEQ ID NO: 43)

SEQ ID NO: 45 prot VH region of MS-Roche#7.11H1 x 7.2L1; protein/1; artificial sequence

QLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSGINAAGF RTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGKGNTHKPYGYVR YFDVWGQGTLVTVSS (SEQ ID NO: 45)

SEQ ID NO: 46 VL region of MS-Roche#3.6H5 x 3.6L2; DNA; artificial sequence GATATCGTGCTGACCCAGAGCCCGGCGACCCTGAGCCTGTCTCCGGGCGAAC GTGCGACCCTGAGCTGCAGAGCGAGCCAGTTTCTTTCTCGTTATTATCTGGCGT GGTACCAGCAGAAACCAGGTCAAGCACCGCGTCTATTAATTTATGGCGCGAGC AGCCGTGCAACTGGGGTCCCGGCGCGTTTTAGCGGCTCTGGATCCGGCACGG ATTTTACCCTGACCATTAGCAGCCTGGAACCTGAAGACTTTGCGGTTTATTATTG CCAGCAGACTTATAATTATCCTCCTACCTTTGGCCAGGGTACGAAAGTTGAAAT TAAACGTACG (SEQ ID NO: 46)

SEQ ID NO:47 prot VL region of MS-Roche#3.6H5 x 3.6L2; protein/1; artificial sequence

DIVLTQSPATLSLSPGERATLSCRASQFLSRYYLAWYQQKPGQAPRLLIYGASSRA TGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQTYNYPPTFGQGTKVEIKRT (SEQ ID NO: 47)

SEQ ID NO: 48 VL region of MS-Roche#3.6H8 x 3.6L2; DNA; artificial sequence GATATCGTGCTGACCCAGAGCCCGGCGACCCTGAGCCTGTCTCCGGGCGAAC GTGCGACCCTGAGCTGCAGAGCCAGCCAGTTTCTTTCTCGTTATTATCTGGCGT GGTACCAGCAGAAACCAGGTCAAGCACCGCGTCTATTAATTTATGGCGCGAGC AGCCGTGCAACTGGGGTCCCGGCGCGTTTTAGCGGCTCTGGATCCGGCACGG ATTTTACCCTGACCATTAGCAGCCTGGAACCTGAAGACTTTGCGGTTTATTATTG CCAGCAGACTTATAATTATCCTCCTACCTTTGGCCAGGGTACGAAAGTTGAAAT TAAACGTACG (SEQ ID NO: 48)

SEQ ID NO: 49 prot VL region of MS-Roche#3.6H8 x 3.6L2; protein/1; artificial sequence

DIVLTQSPATLSLSPGERATLSCRASQFLSRYYLAWYQQKPGQAPRLLIYGASSRA TGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQTYNYPPTFGQGTKVEIKRT (SEQ ID NO: 49)

SEQ ID NO: 50 VL region of MS-Roche#7.4H2 x 7.2L1; DNA; artificial sequence GATATCGTGCTGACCCAGAGCCCGGCGACCCTGAGCCTGTCTCCGGGCGAAC GTGCGACCCTGAGCTGCAGAGCCAGAGCCAGTATGTTGATCGTACTTATCTGGCG TGGTACCAGCAGAAACCAGGTCAAGCACCGCGTCTATTAATTTATGGCGCGAG CAGCCGTGCAACTGGGGTCCCGGCGCGTTTTAGCGGCTCTGGATCCGGCACG GATTTTACCCTGACCATTAGCAGCCTGGAACCTGAAGACTTTGCGACTTATTATT GCCAGCAGATTTATTCTTTTCCTCATACCTTTGGCCAGGGTACGAAAGTTGAAAT TAAACGTACG (SEQ ID NO: 50)

SEQ ID NO: 51 prot VL region of MS-Roche#7.4H2 x 7.2L1; protein/1; artificial sequence

DIVLTQSPATLSLSPGERATLSCRASQYVDRTYLAWYQQKPGQAPRLLIYGASSRA TGVPARFSGSGSGTDFTLTISSLEPEDFATYYCQQIYSFPHTFGQGTKVEIKRT (SEQ ID NO: 51)

SEQ ID NO: 53 prot VL region of MS-Roche#7.9H2 x 7.12 L2; protein/1; artificial sequence

DIVLTQSPATLSLSPGERATLSCRASQRFFYKYLAWYQQKPGQAPRLLISGSSNRA TGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCLQLYNIPNTFGQGTKVEIKRT (SEQ ID NO: 53)

SEQ ID NO: 54 VL region of MS-Roche#7.9H4 x 7.12L2; DNA; artificial sequence

GATATCGTGCTGACCCAGAGCCCGGCGACCCTGAGCCTGTCTCCGGGCGAAC
GTGCGACCCTGAGCTGCAGAGCGAGCCAGCGTTTTTTTTATAAGTATCTGGCGT
GGTACCAGCAGAAACCAGGTCAAGCACCGCGTCTATTAATTTCTGGTTCTTCTA
ACCGTGCAACTGGGGTCCCGGCGCGTTTTAGCGGCTCTGGATCCGGCACGGA
TTTTACCCTGACCATTAGCAGCCTGGAACCTGAAGACTTTGCGGTTTATTATTGC
CTTCAGCTTTATAATATTCCTAATACCTTTGGCCAGGGTACGAAAGTTGAAATTA
AACGTACG (SEQ ID NO: 54)

SEQ ID NO: 55 prot VL region of MS-Roche#7.9H4 x 7.12L2; protein/1; artificial sequence

DIVLTQSPATLSLSPGERATLSCRASQRFFYKYLAWYQQKPGQAPRLLISGSSNRA TGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCLQLYNIPNTFGQGTKVEIKRT (SEQ ID NO: 55)

SEQ ID NO: 56 VL region of MS-Roche#7.11H1 x 7.11L1; DNA; artificial sequence GATATCGTGCTGACCCAGAGCCCGGCGACCCTGAGCCTGTCTCCGGGCGAAC GTGCGACCCTGAGCTGCAGAGCCAGCGAGCCAGCGTATTCTTCGTATTTATCTGGCG TGGTACCAGCAGAAACCAGGTCAAGCACCGCGTCTATTAATTTATGGCGCGAG CAGCCGTGCAACTGGGGTCCCGGCGCGTTTTAGCGGCTCTGGATCCGGCACG GATTTTACCCTGACCATTAGCAGCCTGGAACCTGAAGACTTTGCGACTTATTATT GCCAGCAGGTTTATTCTCCTCCTCCTCATACCTTTGGCCAGGGTACGAAAGTTGAAA TTAAACGTACG (SEQ ID NO: 56)

SEQ ID NO: 57 prot VL region of MS-Roche#7.11H1 x 7.11L1; protein/1; artificial sequence

DIVLTQSPATLSLSPGERATLSCRASQRILRIYLAWYQQKPGQAPRLLIYGASSRAT GVPARFSGSGSGTDFTLTISSLEPEDFATYYCQQVYSPPHTFGQGTKVEIKRT (SEQ ID NO: 57)

SEQ ID NO: 58 VL region of MS-Roche#7.11H1 x 7.2L1; DNA; artificial sequence GATATCGTGCTGACCCAGAGCCCGGCGACCCTGAGCCTGTCTCCGGGCGAAC GTGCGACCCTGAGCTGCAGAGCGAGCCAGTATGTTGATCGTACTTATCTGGCGTGGTACCAGCAGAAACCAGGTCAAGCACCGCGTCTATTAATTTATGGCGCGAG

CAGCCGTGCAACTGGGGTCCCGGCGCGTTTTAGCGGCTCTGGATCCGGCACG
GATTTTACCCTGACCATTAGCAGCCTGGAACCTGAAGACTTTGCGACTTATTATT
GCCAGCAGATTTATTCTTTTCCTCATACCTTTGGCCAGGGTACGAAAGTTGAAAT
TAAACGTACG (SEQ ID NO: 58)

SEQ ID NO: 59 prot VL region of MS-Roche#7.11H1 x 7.2L1; protein/1; artificial sequence

DIVLTQSPATLSLSPGERATLSCRASQYVDRTYLAWYQQKPGQAPRLLIYGASSRA TGVPARFSGSGSGTDFTLTISSLEPEDFATYYCQQIYSFPHTFGQGTKVEIKRT (SEQ ID NO: 59)

SEQ ID NO: 60 HCDR3 region of MS-Roche#3.6H5 x 3.6L2; DNA; artificial sequence

CTTACTCATTATGCTCGTTATTATCGTTATTTTGATGTT (SEQ ID NO: 60)

SEQ ID NO: 61 prot HCDR3 region of MS-Roche#3.6H5 x 3.6L2; protein/1; artificial sequence

LTHYARYYRYFDV (SEQ ID NO: 61)

SEQ ID NO: 62 HCDR3 region of MS-Roche#3.6H8 x 3.6L2; DNA; artificial sequence

CTTACTCATTATGCTCGTTATTATCGTTATTTTGATGTT (SEQ ID NO: 62)

SEQ ID NO: 63 prot HCDR3 region of MS-Roche#3.6H8 x 3.6L2; protein/1; artificial sequence

LTHYARYYRYFDV (SEQ ID NO: 63)

SEQ ID NO: 64 HCDR3 region of MS-Roche#7.4H2 x 7.2L1; DNA; artificial sequence

GGTAAGGGTAATACTCATAAGCCTTATGGTTATGTTCGTTATTTTGATGTT (SEQ ID NO: 64)

SEQ ID NO: 65 prot HCDR3 region of MS-Roche#7.4H2 x 7.2L1; protein/1; artificial sequence

GKGNTHKPYGYVRYFDV (SEQ ID NO: 65)

SEQ ID NO: 66 HCDR3 region of MS-Roche#7.9H2 x 7.12 L2; DNA; artificial sequence

GGTAAGGGTAATACTCATAAGCCTTATGGTTATGTTCGTTATTTTGATGTT (SEQ ID NO: 66)

SEQ ID NO: 67 prot HCDR3 region of#MS-Roche 7.9H2 x 7.12 L2; protein/1; artificial sequence

GKGNTHKPYGYVRYFDV (SEQ ID NO: 67)

SEQ ID NO: 68 HCDR3 region of MS-Roche#7.9H4 x 7.12L2; DNA; artificial sequence

GGTAAGGGTAATACTCATAAGCCTTATGGTTATGTTCGTTATTTTGATGTT (SEQ ID NO: 68)

SEQ ID NO: 69 prot HCDR3 region of MS-Roche#7.9H4 x 7.12L2; protein/1; artificial sequence

GKGNTHKPYGYVRYFDV (SEQ ID NO: 69)

SEQ ID NO: 70 HCDR3 region of MS-Roche#7.11H1 x 7.11L1; DNA; artificial sequence

GGTAAGGGTAATACTCATAAGCCTTATGGTTATGTTCGTTATTTTGATGTT (SEQ ID NO: 70)

SEQ ID NO: 71 prot HCDR3 region of MS-Roche#7.11H1 x 7.11L1; protein/1; artificial sequence

GKGNTHKPYGYVRYFDV (SEQ ID NO: 71)

SEQ ID NO: 72 HCDR3 region of MS-Roche#7.11H1 x 7.2L1; DNA; artificial sequence

GGTAAGGGTAATACTCATAAGCCTTATGGTTATGTTCGTTATTTTGATGTT (SEQ ID NO: 72)

SEQ ID NO: 73 prot HCDR3 region of MS-Roche#7.11H1 x 7.2L1; protein/1; artificial sequence

GKGNTHKPYGYVRYFDV (SEQ ID NO: 73)

SEQ ID NO: 74 LCDR3 region of MS-Roche#3.6H5 x 3.6L2; DNA; artificial sequence

CAGCAGACTTATAATTATCCTCCT (SEQ ID NO: 74)

SEQ ID NO: 75 prot LCDR3 region of MS-Roche#3.6H5 x 3.6L2; protein/1; artificial sequence

QQTYNYPP (SEQ ID NO: 75)

SEQ ID NO: 76 LCDR3 region of MS-Roche#3.6H8 x 3.6L2; DNA; artificial sequence

CAGCAGACTTATAATTATCCTCCT (SEQ ID NO: 76)

SEQ ID NO: 77 prot LCDR3 region of MS-Roche#3.6H8 x 3.6L2; protein/1; artificial sequence

QQTYNYPP (SEQ ID NO: 77)

SEQ ID NO: 78 LCDR3 region of MS-Roche#7.4H2 x 7.2L1; DNA; artificial sequence

CAGCAGATTTATTCTTTTCCTCAT (SEQ ID NO: 78)

SEQ ID NO: 79 prot LCDR3 region of MS-Roche#7.4H2 x 7.2L1; protein/1; artificial sequence

QQIYSFPH (SEQ ID NO: 79)

SEQ ID NO: 80 LCDR3 region of MS-Roche#7.9H2 x 7.12 L2; DNA; artificial sequence

## CTTCAGCTTTATAATATTCCTAAT (SEQ ID NO: 80)

SEQ ID NO: 81 prot LCDR3 region of MS-Roche#7.9H2 x 7.12 L2; protein/1; artificial sequence

LQLYNIPN (SEQ ID NO: 81)

SEQ ID NO: 82 LCDR3 region of MS-Roche#7.9H4 x 7.12L2; DNA; artificial sequence

CTTCAGCTTTATAATATTCCTAAT (SEQ ID NO: 82)

SEQ ID NO: 83 prot LCDR3 region of MS-Roche#7.9H4 x 7.12L2; protein/1; artificial sequence

LQLYNIPN (SEQ ID NO: 83)

SEQ ID NO: 84 LCDR3 region of MS-Roche#7.11H1 x 7.11L1; DNA; artificial sequence

CAGCAGGTTTATTCTCCTCCTCAT (SEQ ID NO: 84)

SEQ ID NO: 85 prot LCDR3 region of MS-Roche#7.11H1 x 7.11L1; protein/1; artificial sequence

QQVYSPPH (SEQ ID NO: 85)

SEQ ID NO: 86 LCDR3 region of MS-Roche#7.11H1 x 7.2L1; DNA; artificial sequence

CAGCAGATTTATTCTTTTCCTCAT (SEQ ID NO: 86)

SEQ ID NO: 87 prot LCDR3 region of MS-Roche#7.11H1 x 7.2L1; protein/1; artificial sequence

QQIYSFPH (SEQ ID NO: 87)

SEQ ID NO: 88 VH region of MS-Roche#7.9H7; DNA; artificial sequence Caggtgcaattggtggaaagcggcggcggcggctggtgcaaccgggcggcagcctgcgctggtgcaaccgggcggcagcctgggtgagcgctatccgggttgagcgctattacctttagcagctatgcgatgagctgggtgcgccaagcccctgggaaagggtctcgagtgggtgagcgctat

taatgcttctggtactcgtacttattatgctgattctgttaagggtcgttttaccatttcacgtgataattcgaaaaacaccctg tatctgcaaatgaacagcctgcgtgcggaagatacggccgtgtattattgcgcgcgtggtaagggtaatactcataag ccttatggttatgttcgttattttgatgtttggggccaaggcaccctggtgacggttagctca (SEQ ID NO: 88)

SEQ ID NO: 89 prot VH region of MS-Roche#7.9H7; protein/1; artificial sequence QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAINAS GTRTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGKGNTHKPYGY VRYFDVWGQGTLVTVSS (SEQ ID NO: 89)

SEQ ID NO: 90 VL region of MS-Roche#7.9H7; DNA; artificial sequence Gatatcgtgctgacccagagcccggcgaccctgagcctgtctccgggcgaacgtgcgaccctgagctgcagagcg agccagagcgtgagcagcagctatctggcgtggtaccagcagaaaccaggtcaagcaccgcgtctattaatttatg gcgcgagcagccgtgcaactggggtcccggcggttttagcggctctggatccggcaccggattttaccctgaccatta gcagcctggaacctgaagactttgcgacttattattgccttcagatttataatatgcctattacctttggccagggtacgaa agttgaaattaaacgtacg (SEQ ID NO: 90)

SEQ ID NO: 91 prot VL region of MS-Roche#7.9H7; protein/1; artificial sequence DIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA TGVPARFSGSGSGTDFTLTISSLEPEDFATYYCLQIYNMPITFGQGTKVEIKRT (SEQ ID NO: 91)

SEQ ID NO: 92 HCDR3 region of MS-Roche#7.9H7; DNA; artificial sequence Ggtaagggtaatactcataagccttatggttatgttcgttattttgatgtt (SEQ ID NO: 92)

SEQ ID NO: 93 prot HCDR3 region of MS-Roche#7.9H7; protein/1; artificial sequence GKGNTHKPYGYVRYFDV (SEQ ID NO: 93)

SEQ ID NO: 94 LCDR3 region of MS-Roche#7.9H7; DNA; artificial sequence Cttcagatttataatatgcctatt (SEQ ID NO: 94)

SEQ ID NO: 95 prot LCDR3 region of MS-Roche#7.9H7; protein/1; artificial sequence

## LQIYNMPI (SEQ ID NO: 95)

Further illustrative sequences are depicted in the appended sequence listing and are also shown in the appended tables, in particular tables 1, 8 and 10.

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The Figures show:

#### Sequence summary of HuCAL®-Fab1 Library Figure 1

The numbering is according to VBASE except the gap in VL $\lambda$  position 9. In VBASE the gap is set at position 10 (Chothia et al., 1992). In the sequence summary all CDR3 residues which were kept constant are indicated. Corresponding sequences employed for the HuCAL-Fab1 library can be found in the appended sequence listing.

A: amino acid sequence

B: DNA sequence

### Fab display vector pMORPH®18 Fab Figure 2

Vector map and DNA sequence including restriction sites

### Fab expression vector pMORPH®x9 Fab Figure 3

Vector map and DNA sequence including restriction sites

### Figure 4 Sequences of the parental Fab fragments MS-Roche-3, MS-Roche-7 and MS-Roche 8

A: amino acid sequence

B: DNA sequence

# Figure 5: Indirect immunofluorescence of amyloid-plagues from a cryostat section of human temporal cortex. The plaques were labeled with MS-R # 3.2 Fab (upper panels) and MS-R # 7.4 Fab (lower panels) at 20 μg/ml (left panels) and 5 μg/ml (right panels) under stringent blocking conditions. Bound MS-R Fab was revealed by goat anti-human-Cy3.

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Figure 6: Indirect immunofluorescence of amyloid-plaques from a cryostat section of human temporal cortex. The plaques were labeled with MS-R # 3.3 IgG1 (upper panels) and MS-R # 7.12 IgG1 (lower panels) at 0.05  $\mu$ g/ml (left panels) and 0.01  $\mu$ g/ml (right panels) under stringent blocking conditions. Bound MS-R IgG1 antibody was revealed by goat anti-human (H+L)-Cy3.

Figure 7: Indirect immunofluorescence of amyloid-plaques from a cryostat section of human temporal cortex using antibodies after final affinity maturation. The plaques were labeled with MS-R # 7.9.H7 IgG1 (MAB 31, top panel), MS-R # 7.11.H1x7.2.L1 IgG1 (MAB 11, middle panel) and MS-R # 3.4.H7, bottom panel). Antibodies were used at 0.05 μg/ml (left panels) and 0.01 μg/ml (right panels) under stringent blocking conditions. Bound MS-R IgG1 antibody was revealed by goat antihuman (H+L)-Cy3.

Scale:  $8,5 \text{ mm} = 150 \mu \text{m}$ .

**Figure 8:** Polymerization Assay. Anti-A $\beta$  antibodies prevent incorporation of biotinylated A $\beta$  into preformed A $\beta$  aggregates.

**Figure 9:** De-polymerization Assay. Anti-Aβ antibodies induce release of biotinylated Aβ from aggregated Aβ.

Figure 10: In vivo decoration of amyloid plaques in an APP/PS2 double transgenic mouse after intravenous injection of 1mg MS-Roche IgG #7.9.H2 x 7.12.L2. After three days the mouse was perfused with phosphate-buffered saline and sacrificed. The presence of human IgG bound to amyloid plaques was revealed by confocal microscopy after labelling cryostat sections from the frontal cortex with a goat anti-human IgG-Cy3 conjugate (panel B). The same section was counterstained with an anti-Abeta mouse monoclonal antibody (BAP-2-Alexa488 conjugate, panel A) to visualize the position of amyloid plaques. Individual red

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(panel B) and green (panel A) channels, merged image (panel D) and colocalized (pancel C) signals are shown.

Scale:  $1 \text{ cm} = 50 \mu\text{m}$ 

Figure 11: In vivo decoration of amyloid plaques in an APP/PS2 double transgenic mouse after intravenous injection of 1mg MS-Roche IgG #7.9.H4 x 7.12.L2. Experimental conditions and staining procedure were identical to those described in the legend of figure 10.

Scale:  $1.6 \text{ cm} = 50 \mu\text{m}$ 

Figure 12: In vivo decoration of amyloid plaques in an APP/PS2 double transgenic mouse after intravenous injection of 1mg MS-Roche IgG #7.11.H1 x 7.2.L1 (MAB 11). Experimental conditions and staining procedure were identical to those described in the legend of figure 10.

Scale:  $1.4 \text{ cm} = 70 \mu\text{m}$ 

Figure 13: In vivo decoration of amyloid plaques in an APP/PS2 double transgenic mouse after intravenous injection of 2 mg MS-Roche IgG #7.9.H7 (MAB 31) at day 0, 3, and 6. After nine days the mouse was perfused with phosphate-buffered saline and sacrificed. The presence of human IgG bound to amyloid plaques was revealed by confocal microscopy after labelling cryostat sections from the frontal cortex with a goat antihuman IgG-Cy3 conjugate (panel B). The same section was counterstained with an anti-Abeta mouse monoclonal antibody (BAP-2-Alexa488 conjugate, panel A) to visualize the position of amyloid plaques. Individual red (panel B) and green (panel A) channels, merged image (panel D) and colocalized (panel C) signals and are shown.

Scale: 1.6 cm = 80  $\mu$ m (panels A, B, C); 1.0 cm = 50  $\mu$ m (panel D)

Figure 14: In vivo decoration of amyloid plaques in an APP/PS2 double transgenic mouse after intravenous injection of 2 mg MS-Roche IgG #7.11.H1 x 7.2.L1 (MAB 11) at day 0, 3 and 6. Experimental conditions and

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staining procedure were identical to those described in the legend of figure 13.

Scale: 1.6 cm = 80  $\mu$ m

Figure 15: Binding analysis of anti-Aβ antibodies to cell surface APP. Antibody binding to human APP-transfected HEK293 cells and non-transfected control cells was analyzed by flow cytometry.

The examples illustrate the invention.

# Example 1: Construction and Screening of a Human Combinatorial Antibody Library (HuCAL®-Fab 1)

Cloning of HuCAL®-Fab 1

HuCAL<sup>®</sup>-Fab 1 is a fully synthetic, modular human antibody library in the Fab antibody fragment format. HuCAL<sup>®</sup>-Fab 1 was assembled starting from an antibody library in the single-chain format (HuCAL<sup>®</sup>-scFv; *Knappik*,(2000), *J. Mol. Biol.* 296, 57-86).

 $V\lambda$  positions 1 and 2. The original HuCAL® master genes were constructed with their authentic N-termini: VL $\lambda$ 1: QS (CAGAGC), VL $\lambda$ 2: QS (CAGAGC), and VL $\lambda$ 3: SY (AGCTAT). Sequences containing these amino acids are shown in WO 97/08320. During HuCAL® library construction, the first two amino acids were changed to DI to facilitate library cloning (EcoRI site). All HuCAL® libraries contain VL $\lambda$  genes with the EcoRV site GATATC (DI) at the 5'-end. All HuCAL® kappa genes (master genes and all genes in the library) contain DI at the 5'-end (figure 1 A and B).

VH position 1. The original HuCAL® master genes were constructed with their authentic N-termini: VH1A, VH1B, VH2, VH4, and VH6 with Q (=CAG) as the first amino acid and VH3 and VH5 with E (=GAA) as the first amino acid. Sequences containing these amino acids are shown in WO 97/08320. During cloning of the HuCAL®-Fab1 library, amino acid at position 1 of VH was changed to Q (CAG) in all VH genes (figure 1 A and B).

Design of the CDR libraries

 $V\kappa1/V\kappa3$  position 85. Because of the cassette mutagenesis procedure used to introduce the CDR3 library (*Knappik, (2000), loc. cit.*), position 85 of V $\kappa$ 1 and V $\kappa$ 3 can be either T or V. Thus, during HuCAL®-scFv1 library construction, position 85 of V $\kappa$ 1 and V $\kappa$ 3 was varied as follows: V $\kappa$ 1 original, 85T (codon ACC); V $\kappa$ 1 library, 85T or 85V (TRIM codons ACT or GTT); V $\kappa$ 3 original, 85V (codon GTG); V $\kappa$ 3 library, 85T or 85V (TRIM codons ACT or GTT); the same applies to HuCAL®-Fab1.

CDR3 design. All CDR3 residues, which were kept constant, are indicated in figure 1 A and B.

CDR3 length. The designed CDR3 length distribution is as follows. Residues, which were varied are shown in brackets (x) in figure 1. V kappa CDR3, 8 amino acid residues (position 89 to 96) (occasionally 7-10 residues), with Q89, S90, and D92 fixed; and VH CDR3, 5 to 28 amino acid residues (position 95 to 102) (occasionally 4-28), with D101 fixed.

HuCAL®-Fab 1 was cloned into a phagemid expression vector pMORPH®18\_Fab1 (figure 2). This vector comprises the Fd fragment with a phoA signal sequence fused at the C-terminus to a truncated gene III protein of filamentous phage, and further comprises the light chain VL-CL with an ompA signal sequence. Both chains are under the control of the lac operon. The constant domains Cλ Cκ and CH1 are synthetic genes fully compatible with the modular system of HuCAL® (*Knappik*, (2000), loc. cit.).

The whole VH-chain (Munl/Styl-fragment) was replaced by a 1205 bp dummy fragment containing the  $\beta$ -lactamase transcription unit (bla), thereby facilitating subsequent steps for vector fragment preparation and allowing for selection of complete VH removal.

After VH-replacement, VL $\lambda$  was removed by *EcoRI/DrallI* and VL $\kappa$  by *EcoRI/BsIWI* and replaced with bacterial alkaline phosphatase (bap) gene fragment (1420 bp).

As the variability of the light chains is lower than that of the heavy chains, cloning was started with the light chain libraries. The  $VL_{\lambda}$  and  $VL_{\kappa}$  light chain libraries diversified in L-CDR3, which were generated for the HuCAL®-scFv library (Knappik, (2000), loc. cit.) were also used for cloning of HuCAL®-Fab1. In case of  $\lambda$ they consisted of the  $\lambda$ 1-,  $\lambda$ 2- and  $\lambda$ 3-HuCAL®-framework and had a total variability of 5.7

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x 10<sup>6</sup>. VL<sub>λ</sub> fragmentswere amplified by 15 PCR cycles (Pwo-polymerase) with 5'-GTGGTGGTTCCGATATC-3' 5′-(SEQ ID NO: 28) AGCGTCACACTCGGTGCGGCTTTCGGCTGGCCAAGAACGGTTA-3' (SEQ ID NO: 29). PCR-products were digested with EcoRV/DrallI and gel-purified. In case of the  $VL_{\lambda}$ -library, the bap-dummy was removed by EcoRV/DraIII from the library vector. 2 µg of gelpurified vector were ligated with a 3-fold molar excess of VL<sub>λ</sub>chains for 16 h at 16°C, and the ligation mixtures were electroporated in 800 µl E. coli TOP10F cells (Invitrogen), vielding altogether 4.1 x 108 independent colonies. The transformants were amplified about 2000-fold in 2 x YT/1% glucose/34 µg/ml chloramphenicol/100 µg/ml ampicillin, harvested and stored in 20% (w/v) glycerol at −80°C.

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The  $\kappa$  libraries comprise the  $\kappa$ 1-,  $\kappa$ 2-,  $\kappa$ 3- and  $\kappa$ 4-HuCAL® master genes with a total variability of 5.7 x 10<sup>6</sup>. VL $_{\kappa}$ -chains were obtained by restriction digest with *EcoRV/Bs/WI* and gel-purified. In case of the VL $_{\kappa}$ -library, the bap-dummy was removed by *EcoRV/Bs/WI* from the library vector. 2  $\mu$ g of gel-purified vector were mixed with a 5-fold molar excess of VL $_{\kappa}$ -chains. Ligation and transformation into *E. coli* TOP10F cells (Invitrogen) was performed as described for VL $_{\lambda}$ -chains, yielding altogether 1.6 x 10<sup>8</sup> independent colonies.

DNA of the two light chain libraries was prepared and the bla-dummy was removed by Munl/Styl, thereby generating the two vectors for insertion of the VH sub-libraries. The VH libraries of  $HuCAL^{\circledast}$ -scFv were used for the generation of  $HuCAL^{\circledast}$ -Fab1. The VH libraries of  $HuCAL^{\circledast}$ -scFv consist of the master genes VH1A/B-6 diversified with two VH-CDR3 trinucleotide library cassettes differing in CDR3 length separately, and each VH-library combined with the  $VL_{\kappa^-}$  and with the  $VL_{\lambda^-}$ -library. For the generation of the  $HuCAL^{\circledast}$ -Fab1 DNA from these VH-libraries was prepared preserving the original variability. The DNA was digested with Munl/Styl and gelpurified. A 5-fold molar excess of the VH-chains was ligated with 3  $\mu$ g of the  $VL_{\lambda^-}$ -library vector for 4 h at 22°C. The ligation mixtures were electroporated for each vector in 1200  $\mu$ l E. coli TOP10F cells (Invitrogen), yielding altogether 2.1 x  $10^{10}$  independent colonies. The transformants were amplified about 4000-fold in 2 x YT/1% glucose/34  $\mu$ g/ml chloramphenicol/10  $\mu$ g/ml tetracycline, harvested and stored in 20% (w/v) glycerol at -80°C.

As quality control the light chain and heavy chain of single clones was sequenced with 5'-CAGGAAACAGCTATGAC-3' (SEQ ID NO: 30) and 5'-TACCGTTGCTCTTCACCCC-3' (SEQ ID NO: 31), respectively.

### Phagemid rescue, phage amplification and purification

HuCAL®-Fab 1 was amplified in 2 x TY medium containing 34 μg/ml chloramphenicol, 10 μg/ml tetracycline and 1 % glucose (2 x TY-CG). After helper phage infection (VCSM13) at 37°C at an OD $_{600}$  of about 0.5, centrifugation and resuspension in 2 x TY / 34 μg/ml chloramphenicol / 50 μg/ml kanamycin cells were grown overnight at 30°C. Phage were PEG-precipitated from the supernatant (Ausubel, (1998), Current protocols in molecular biology. John Wiley & Sons, Inc., New York, USA), resuspended in PBS/20% glycerol and stored at -80°C. Phage amplification between two panning rounds was conducted as follows: mid-log phase TG1-cells were infected with eluted phage and plated onto LB-agar supplemented with 1% of glucose and 34 μg/ml of chloramphenicol. After overnight incubation at 30°C colonies were scraped off, adjusted to an OD $_{600}$  of 0.5 and helper phage added as described above.

## Example 2: Solid phase panning

Wells of MaxiSorp<sup>TM</sup> microtiterplates F96 (Nunc) were coated with 100  $\mu$ l 2.5  $\mu$ M human A $\beta$  (1-40) peptide (Bachem) dissolved in TBS containing NaN $_3$  (0.05% v/v) and the sealed plate was incubated for 3 days at 37 °C where the peptide is prone to aggregate on the plate. After blocking with 5% non-fat dried milk in TBS, 1–5 x 10<sup>12</sup> HuCAL<sup>®</sup>-Fab phage purified as above were added for 1h at 20°C. After several washing steps, bound phages were eluted by pH-elution with 500 mM NaCl, 100 mM glycin pH 2.2 and subsequent neutralisation with 1M TRIS-Cl pH 7. Three rounds of panning were performed with phage amplification conducted between each round as described above, the washing stringency was increased from round to round.

### **Example 3: Subcloning of selected Fab fragments for expression**

The Fab-encoding inserts of the selected HuCAL®-Fab fragments were subcloned into the expression vector pMORPH®x7\_FS to facilitate rapid expression of soluble Fab. The DNA preparation of the selected HuCAL®-Fab clones was digested with Xbal/EcoRI, thus cutting out the Fab encoding insert (ompA-VL and phoA-Fd). Subcloning of the purified inserts into the Xbal/EcoRI cut vector pMORPH®x7, previously carrying a scFv insert, leads to a Fab expression vector designated pMORPH®x9\_Fab1 (figure 3). Fabs expressed in this vector carry two C-terminal tags (FLAG and Strep) for detection and purification.

# Example 4: Identification of $A\beta$ -binding Fab fragments by ELISA

Wells of Maxisorp<sup>TM</sup> microtiterplates F384 (Nunc) were coated with 20 $\mu$ I 2.5  $\mu$ M human A $\beta$  (1-40) peptide (Bachem) dissolved in TBS containing NaN<sub>3</sub> (0.05% v/v) and the sealed plate was incubated for 3 days at 37 °C, where the peptide is prone to aggregate on the plate. Expression of individual Fab was induced with 1 mM IPTG for 16 h at 22°C. Soluble Fab was extracted from *E. coli* by BEL lysis (boric acid, NaCl, EDTA and lysozyme containing buffer pH 8) and used in an ELISA. The Fab fragment was detected with an alkaline phosphatase-conjugated goat anti-Fab antibody (Dianova/Jackson Immuno Research). After excitation at 340 nm the emission at 535 nm was read out after addition of AttoPhos fluorescence substrate (Roche Diagnostics).

# **Example 5: Optimization of antibody fragments**

In order to optimize the binding affinity of the selected A $\beta$  binding antibody fragments, some of the Fab fragments, MS-Roche-3 (MSR-3), MS-Roche-7 (MSR-7) and MS-Roche-8 (MSR-8) (figure 4), were used to construct a library of Fab antibody fragments by replacing the parental VL  $\kappa$ 3 chain by the pool of all kappa chains  $\kappa$ 1-3 diversified in CDR3 from the HuCAL<sup>®</sup> library (*Knappik et al., 2000*).

The Fab fragments MS-Roche-3, 7 and 8 were cloned via *Xbal/EcoRI* from pMORPH<sup>®</sup>x9 \_FS into pMORPH<sup>®</sup>18, a phagemid-based vector for phage display of

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Fab fragments, to generate pMORPH<sup>®</sup>18\_Fab1 (figure 2). A kappa chain pool was cloned into pMORPH<sup>®</sup>18 Fab1 via *Xbal/SphI* restriction sites.

The resulting Fab optimization library was screened by panning against aggregated human A $\beta$  (1-40) peptide coated to a solid support as described in example 2. Optimized clones were identified by koff-ranking in a Biacore assay as described in

Example 8. The optimized clones MS-Roche-3.2, 3.3, 3.4, 3.6, 7.2, 7.3, 7.4, 7.9, 7.11, 7.12, 8.1, 8.2, were further characterized and showed improved affinity and biological activity compared to the starting fragment MS-Roche-3, MS-Roche-7 and MS-Roche-8 (figure 4). The CDRs listed refer to the HuCAL® consensus-based antibody gene VH3kappa3. The Fab fragment MS-Roche-7.12 was obtained by cloning the HCDR3 of parental clone MS-R 7 into a HuCAL®-Fab library, carrying diversity in all 6 CDR regions using a design procedure identical with that for CDR3 cassettes described in Knappik *et al., 2000.* The library cassettes were designed strongly biased for the known natural distribution of amino acids and following the concept of canonical CDR conformations established by Allazikani (Allazikani et al., 1997). However in contrast to the HuCAL® master genes, the clone MS-Roche 7.12 contains amino acid S at position 49 of the VL chain (see appended table 1).

The optimized Fabs after the first affinity maturation round showed improved characteristics over the starting MS-Roche-3, MS-Roche-7 and MS-Roche-8 clones (Figure 4). The binding affinities of the maturated Fabs to A $\beta$ 1-40 and A $\beta$ 1-42 were significantly increased yielding  $K_D$  values in the range of 22 – 240 nM in comparison to 850 – 1714 nM of the parental clones (Table 3). Immunohistochemistry analysis of amyloid plaques in human AD brain sections also showed a significantly increased staining profile of the maturated clones, i. e. better signal to background ratios were obtained and positive plaque staining was detected at relatively low concentrations of the maturated Fabs (Figure 5).

For further optimization, the VH CDR2 regions and the VL CDR1 regions of a set of antibody fragments derived from L-CDR3 optimized MS-Roche-3, -7 and -8 (table 1; figure 4) were optimized by cassette mutagenesis using trinucleotide-directed mutagenesis (Virnekäs *et al.*, 1994). Therefore, a trinucleotide-based HCDR2

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cassette and a trinucleotide-based LCDR1 cassette were constructed using a design procedure identical with that for CDR3 cassettes described in Knappik *et al.*, 2000. The library cassettes were designed strongly biased for the known natural distribution of amino acids and following the concept of canonical CDR conformations established by Allazikani (Allazikani et al., 1997). The protocol used for the optimization of the initial selected antibody fragments would mimic the process of affinity maturation by somatic hypermutation observed during the natural immune response.

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The resulting libraries were screened separately as described above leading to optimized clones either in the H-CDR2 or in the L-CDR1 region. All clones were identified as above by an improved koff towards A $\beta$ 1-40-fibers after a koff-ranking in the Biacore and showed improved affinity either to A $\beta$ 1-40 or A $\beta$ -42 or both when compared to the corresponding parent clone (Table 3). Table 1 contains the sequence characteristics of the parental as well as sequences of the optimized clones. The CDRs listed refer to the HuCAL® consensus-based antibody gene VH3kappa3.

For example, the affinity of the MS-Roche-7 parental Fab towards Ab1-40 was improved over 35-fold from 1100 nM to 31 nM after L-CDR3 optimization (MS-Roche-7.9) and further improved to 5 nM after H-CDR2 optimization (MS-Roche-7.9H2) as illustrated in Table 3.

The H-CDR2 and L-CDR1 optimization procedure not only increased the affinity but also resulted for some of the clones in a significantly improved staining of amyloid plaques in AD brain section, as particularly seen with MS-Roche 7.9H2 and 7.9H3.

Table

Binder name	L-CDR1	pos.49	L-CDR2	pos. 85	L-CDR3	H-CDR1	pos.47	H-CDR2	H-CDR3
MS-Roche #3	RASQSVSSSYLA	>	GASSRAT	>	QQVYNPPV	GFTFSSYAMS	M	AISGSGGSTYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.1	RASQSVSSSYLA	>	GASSRAT	L	QQVYSVPP	GFTFSSYAMS	M	AISGSGGSTYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.2	RASQSVSSSYLA	>	GASSRAT	>	QQIYSYPP	GFTFSSYAMS	W	AISGSGGSTYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.3	RASGSVSSSYLA	>	GASSRAT	^	HOMSSYPP	GFTFSSYAMS	W	AISGSGGSTYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4	RASQSVSSSYLA	>	GASSRAT	<b>-</b>	QQTYDYPP	GFTFSSYAMS	W	AISGSGGSTYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.5	RASQSVSSSYLA	>	GASSRAT	Н	QQIYDYPP	GFTFSSYAMS	W	AISGSGGSTYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.6	RASQSVSSSYLA	>	GASSRAT	۸	<b>GQTYNYPP</b>	GFTFSSYAMS	≯	AISGSGGSTYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.2.H1	RASQSVSSSYLA	>	GASSRAT	^	QQIYSYPP	GFTFSSYAMS	W	AISEHGLNIYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.2.H2	RASQSVSSSYLA	>	GASSRAT	>	QQIYSYPP	GFTFSSYAMS	W	AISQRGQFTYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.3.H1	RASQSVSSSYLA	>	GASSRAT	>	HQMSSYPP	GFTFSSYAMS	W	VISEKSRFIYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.3.H2	RASQSVSSSYLA	>	GASSRAT	>	HQMSSYPP	GFTFSSYAMS	M	VISQESQYKYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.3.H3	RASOSVSSSYLA	>	GASSRAT	>	HQMSSYPP	GFTFSSYAMS	8	AISQNGFHIYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4.H1	RASQSVSSSYLA	>	GASSRAT	⊢	QQTYDYPP	GFTFSSYAMS	W	AISETSIRKYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4.H2	RASQSVSSSYLA	>	GASSRAT	1	QQTYDYPP	GFTFSSYAMS	M	VIDMVGHTYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4.H3	RASQSVSSSYLA	>	GASSRAT	<b>-</b>	QQTYDYPP	GFTFSSYAMS	W	VISQTGRKIYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4.H4	RASQSVSSSYLA	>	GASSRAT	1	QQTYDYPP	GFTFSSYAMS	≥	AISETGMHIYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4.H5	RASQSVSSSYLA	>	GASSRAT	Τ	QQTYDYPP	GFTFSSYAMS	×	VISQVGAHIYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4.H6	RASQSVSSSYLA	>	GASSRAT	⊢	QQTYDYPP	GFTFSSYAMS	W	AISESGWSTYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4.H7	RASQSVSSSYLA	>	GASSRAT	1	QQTYDYPP	GFTFSSYAMS	Μ	VISETGKNIYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4.H8	RASQSVSSSYLA	>	GASSRAT	ı	QQTYDYPP	GETFSSYAMS	W	AISEHGRFKYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4.H9	RASQSVSSSYLA	<b>&gt;</b>	GASSRAT	<b>—</b>	QQTYDYPP	GETESSYAMS	×	AISESSKNKYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4.H10	RASQSVSSSYLA	<b>&gt;</b> -	GASSRAT	1	QQTYDYPP	GFTFSSYAMS	Μ	AISESGRGKYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4.H11	RASQSVSSSYLA	>	GASSRAT	_	QQTYDYPP	GFTFSSYAMS	W	AISEFGKNIYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4.H12	RASQSVSSSYLA	>	GASSRAT	_	QQTYDYPP	GFTFSSYAMS	Μ	VISQTGQNIYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4.H13	RASQSVSSSYLA	>	GASSRAT	<b>–</b>	QQTYDYPP	GFTFSSYAMS	W	AISEQGRNIYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4.H14	RASQSVSSSYLA	>	GASSRAT	_	QQTYDYPP	GFTFSSYAMS	W	AISESGQYKYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4.H16	RASQSVSSSYLA	<b>&gt;</b>	GASSRAT	-	QQTYDYPP	GFTFSSYAMS	×	AISESGVNIYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4.H17	RASQSVSSSYLA	<b>&gt;</b>	GASSRAT	T	QQTYDYPP	GFTFSSYAMS	≥	AISEFGQFIYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4.H18	RASQSVSSSYLA	Y	GASSRAT	T	QQTYDYPP	GFTFSSYAMS	>	AISQQSNFIYYADSVKG	LTHYARYYRYFDV

LTHYARYYRYFDV	GKGNTHKPYGYVRYF DV	GKGNTHKPYGYVRYK DV	GKGNTHKPYGYVRYF DV	GKGNTHKPYGYVRYF DV	GKGNTHKPYGYVRYF DV	GKGNTHKPYGYVRYF DV																		
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MS-Roche #3.4.L7	MS-Roche #3.4.L8	MS-Roche #3.4.L9	MS-Roche #3.4.L11	MS-Roche #3.6.H1	MS-Roche #3.6.H2	MS-Roche #3.6.H3	MS-Roche #3.6.H4	MS-Roche #3.6.H5	MS-Roche #3.6.H6	MS-Roche #3.6.H8	MS-Roche #3.6.L1	MS-Roche #3.6.L2	MS-Roche #7	MS-Roche #7.1	MS-Roche #7.2	MS-Roche #7.3	MS-Roche #7.4	MS-Roche #7.5	MS-Roche #7.6	MS-Roche #7.7	MS-Roche #7.8	MS-Roche #7.9	MS-Roche #7.10	MS-Roche #7.11

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MS-Roche #7.12	MS-Roche #7.13	MS-Roche #7.2.H1	MS-Roche #7.2.H2	MS-Roche #7.2.H3	MS-Roche #7.2.H4	MS-Roche #7.2.H5	MS-Roche #7.2.H6	MS-Roche #7.2.H7	MS-Roche #7.2.H8	MS-Roche #7.2.L1	MS-Roche #7.2.L2	MS-Roche #7.2.L4	MS-Roche #7.3.H1	MS-Roche #7.3.L1	MS-Roche #7.4.H1	MS-Roche #7.4.H2	MS-Roche #7.9.H1	MS-Roche #7.9.H2	MS-Roche #7.9.H3	

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GASSRAT	GASSRAT	GASSRAT	GASSRAT	GASSRAT	GASSRAT	GASSRAT	GASSRAT	GASSRAT	GASSRAT	GASSRAT	GASSRAT	GASSRAT	GASSRAT	GSSNRAT	GSSNRAT	GSSNRAT	GSSNRAT	GSSNRAT	GSSNRAT
>	>	>	<b>&gt;</b>	>	>	>	>	>	>	>	>	<b>&gt;</b>	>	S	S	S	တ	S.	S
RASQSVSSSYLA	RASQSVSSSYLA	RASQRLSPRYLA	RASQYLHKRYLA	RASQSVSSSYLA	RASQSVSSSYLA	RASQSVSSSYLA	RASQSVSSSYLA	RASQSVSSSYLA	RASQSVSSSYLA	RASQSVSSSYLA	RASQSVSSSYLA	RASQSVSSSYLA	RASQRILRIYLA	RASQYVFRRYLA	RASQYVFRRYLA	RASQRFFYKYLA	RASQFVRRGFLA	RASQRLKRSYLA	RASQRLKRSYLA
MS-Roche #7.9.H4	MS-Roche #7.9.H5	MS-Roche #7.9.L1	MS-Roche #7.9.L 2	MS-Roche #7.9.H6	MS-Roche #7.9.H7	MS-Roche #7.9.H8	MS-Roche #7.9.H9	MS-Roche #7.11.H1	MS-Roche #7.11.H2	MS-Roche #7.11.H3	MS-Roche #7.11.H4	MS-Roche #7.11.H5	MS-Roche # 7.11.L1	MS-Roche #7.12.H1	MS-Roche #7.12.L1	MS-Roche #7.12.L2	MS-Roche #7.12.L3	MS-Roche #7.12.L4	MS-Roche #7.12.L5

	V LQLYNIPN T QQLSSFPP T QQLSSFPP	0.0	3 3 3	NISGSGSSTYYADSVKG NISGSGSSTYYADSVKG	GKGNTHKPYGYVRYF DV GKGNTHKPYGYVRYF DV LLSRGYNGYYHKFDV
RASQWIRKTYLA S GSSNRAT V LQLYNIPN DASOSKSSSVI A Y CASSDAT T OOI SSEPP	V LQLYNIPY T QQLSSFF T QQLSNYF	0 0	× ×	NISGSGSSTYYADSVKG	GKGNTHKPYGYVRYF DV LLSRGYNGYYHKFDV
DASOS/SSSVI Y CASSDAT T OOLSSEPP	T QQLSSFP T QQLSNYF		W	OWGONYTGOOGIA	LLSRGYNGYYHKFDV
ואיסמסטורים ואיסטטורים ואיטטטורים ואיטטטורים ואיטטטוריט	T QQLSNYF		141	MISSOSSI I TADSVNG	
MS-Roche #8.1 RASQSVSSSYLA Y GASSRAT T QQLSNYPP GFTFSSYAMS			۸۸	AISGSGGSTYYADSVKG	LLSRGYNGYYHKFDV
MS-Roche #8.2 RASQSVSSSYLA Y GASSRAT T QQLSSYPP GFTFSSYAMS	T QQLSSYF		Μ	AISGSGGSTYYADSVKG	LLSRGYNGYYHKFD!
MS-Roche #8.1.H1 RASQSVSSSYLA Y GASSRAT T QQLSNYPP GFTFSSYAMS	T QQLSNYF		M	AISRSGSNIYYADSVKG	LLSRGYNGYYHKFDV
MS-Roche #8.2.H1 RASQSVSSSYLA Y GASSRAT T QQL.SSYPP GFTFSSYAMS	T QQLSSYF		Μ	AISITGRRKYYADSVKG	LLSRGYNGYYHKFDV
MS-Roche #8.2.H2 RASQSVSSSYLA Y GASSRAT T QQLSSYPP GFTFSSYAMS	T QQLSSYF		Α	AISRTGSKTYYADSVKG	LLSRGYNGYYHKFDV
MS-Roche #8.2.H4 RASQSVSSSYLA Y GASSRAT T QQLSSYPP GFTFSSYAMS	T QQLSSYF		Μ	ATSVKGKTYYADSVKG	LLSRGYNGYYHKFDV
MS-Roche #8.2.L1 RASQRVSGRYLA Y GASSRAT T QQLSSYPP GFTFSSYAMS	T QQLSSYF		Μ	AISGSGGSTYYADSVKG	LLSRGYNGYYHKFDV

Sequences belonging to  $V_{H}3$  and  $V_{K}3$  HuCAL consensus sequences see Figure 1 A

### Example 6

Construction of HuCAL® immunoglobulin expression vectors

Heavy chain cloning. The multiple cloning site of pcDNA3.1+ (invitrogen) was removed (Nhel/Apal), and a stuffer compatible with the restriction sites used for HuCAL® design was inserted for the ligation of the leader sequences (Nhel/EcoRI), VH-domains (Munl/), and the immunoglobulin constant regions (Blpl/Apal). The leader sequence (EMBL 83133) was equipped with a Kozak sequence (Kozak, 1987). The constant regions of human IgG (PIR A02146), IgG4 (EMBL K01316), and serum IgA1 (EMBL J00220) were dissected into overlapping oligonucleotides with length of about 70 bases. Silent mutations were introduced to remove restriction sites non-compatible with the HuCAL® design. The oligonucleotides were spliced by overlap extension-PCR.

During sub-cloning from Fab into IgG, the VH DNA sequence of the Fab is cut out via Mfe I / Blp I and ligated into the IgG vector opened via EcoR I / Blp I. EcoR I (g/aattc) and Mfe I (c/aattg) share compatible cohesive ends (aatt) and the DNA sequence of the original Mfe I site in the Fab changes from: c/aattg to: g/aattg after ligation into the IgG expression vector, thereby destroying both Mfe I and EcoR I site, and thus also leading to an amino acid change from Q (codon: caa) to E (codon: gaa).

Light chain cloning. The multiple cloning site of pcDNA3.1/Zeo+ (Invitrogen) was replaced by two different stuffers. The  $\kappa$ -stuffer provided restriction sites for insertion of a  $\kappa$ -leader (*Nhel/EcoRV*), HuCAL®-scFv V $\kappa$ -domains (*EcoRV/BsiWI*), and the  $\kappa$ -chain constant region (*BsiWI/ApaI*). The corresponding restriction sites in the  $\lambda$ -stuffer were *Nhel/EcoRV* ( $\lambda$ -leader), *EcoRV/HpaI* (V $\lambda$ -domains), and *HpaI/ApaI* ( $\lambda$ -chain constant region). The  $\kappa$ -leader (EMBL Z00022) as well as the  $\lambda$ -leader (EMBL J00241) were both equipped with Kozak sequences. The constant regions of the human  $\kappa$ - (EMBL L00241) and  $\lambda$ -chain (EMBL M18645) were assembled by overlap extension-PCR as described above.

Generation of IgG-expressing CHO-cells. CHO-K1 cells were co-transfected with an equimolar mixture of IgG heavy and light chain expression vectors. Double-resistant

transfectants were selected with 600  $\mu$ g/ml G418 and 300  $\mu$ g/ml Zeocin (Invitrogen) followed by limiting dilution. The supernatant of single clones was assessed for IgG expression by capture-ELISA. Positive clones were expanded in RPMI-1640 medium supplemented with 10% ultra-low IgG-FCS (Life Technologies). After adjusting the pH of the supernatant to 8.0 and sterile filtration, the solution was subjected to standard protein A column chromatography (Poros 20 A, PE Biosystems).

### **Example 7: Pepspot analysis with decapeptides**

The following aminoacid sequence encompassing A $\beta$  (1-42) was divided into 43 overlapping decapeptides with a frameshift of 1 aminoacid.

ISEVKM<sup>1</sup>DAEF RHDSGYEVHH QKLVFFAEDV GSNKGAIIGL MVGGVVI<sup>42</sup>ATV IV (SEQ ID NO: 414). Accordingly, DAEF RHDSGYEVHH QKLVFFAEDV GSNKGAIIGL MVGGVVIA (SEQ ID NO: 27) as enclosed represents amino acids 1 to 42 of Aβ4/β-A4 peptide.

The 43 decapeptides were synthesized with N-terminal acetylation and C-terminal covalent attachment to a cellulose sheet ("pepspot") by a commercial supplier (Jerini BioTools, Berlin). The cellulose sheet is incubated for 2 hours on a rocking platform with monoclonal antibody (2 µg/ml) in blocking buffer (50 mM TrisHCl, 140 mM NaCl, 5 mM NaEDTA, 0.05% NP40 (Fluka), 0.25% gelatine (Sigma), 1% bovine serum albumine fraction V (Sigma), pH 7.4). The sheet is washed 3 times 3 minutes on a rocking platform with TBS (10 mM Tris.HCl, 150 mM NaCl, pH 7.5). It is then wetted with cathode buffer (25 mM Tris base, 40 mM 6-Aminohexane acid, 0.01% SDS, 20% methanol) and transfered to a semi-dry blotting stack with the peptide side facing a PVDF membrane (Biorad) of equal size.

The semi-dry blotting stack consists out of freshly wetted filter papers (Whatman No.3) slightly larger than the peptide sheet:

3 papers wetted with Cathode buffer

the peptide sheet

- a sheet of PVDF membrane wetted with methanol
- 3 papers wetted with Anode buffer 1 (30mM Tris base, 20% methanol)
- 3 papers wetted with Anode buffer 2 (0.3 mM Tris base, 20% methanol)

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The transfer is conducted at a current density between Cathode and Anode of 0.8 mA/cm<sup>2</sup> for 40 minutes which is sufficient to elute most of the antibody from the cellulose sheet and deposit it on the PVDF membrane. The PVDF membrane is then exchanged for a 2<sup>nd</sup> PVDF membrane and transferred for another 40 minutes to ensure complete elution from the cellulose sheet.

The PVDF membrane is immersed in blocking buffer for 10 minutes. Then HRP-labeled anti-human Ig H+L (Pierce) is added at 1:1000 dilution and the membrane is incubated on a rocking platform for 1 hour. It is washed 3x10 minutes with TBST (TBS with 0.005% Tween20). Color is developed by immersing the membrane into a solution made of 3 mg 4-chloronaphthol dissolved in 9 ml methanol with 41 ml PBS (20 mM Na-phosphate, 150 mM NaCl, pH 7.2) an 10 µl 30% hydrogen peroxide (Merck). After the development of blue-black spots the membrane is washed extensively with water and dried.

The assignment of antibody-reactive pepspots is made by visual inspection through a transparent spot matrix. The epitopes of the antibody in question is defined as the minimal aminoacid sequence in reactive peptides. For comparison mouse monoclonal antibodies (BAP-2, BAP-1, BAP-17 BAP-21, BAP-24, and 4G8) are analyzed in the same way, except using HRP-labeled anti-mouse Ig instead of anti-human Ig.

It is of note that affinity maturation and conversion of the monovalent Fab fragments into full-length IgG1 antibodies results usually in some broadening of the epitope recognition sequence as indicated by pepspot and ELISA analyses. This may be related to the recruitment of more contact points in the antibody-antigen interaction area as a consequence of the affinity maturation or to a stronger binding to the minimal epitope such that also weak interactions with adjacent amino acid can be detected. The latter may be the case when Aβ-derived peptides are probed with full-length IgG antibodies. As illustrated in Table 2 for the pepspot analysis, the recognition sequences of the N-terminal and middle epitopes are extended by up to three amino acids when parent Fabs and corresponding fully maturated IgG antibodies are compared. However, it has to be kept in mind that the decapeptides are modified for covalent attachment at the C-terminal amino acid and this amino acid may therefore not easily be accessible to the full-length antibody due to steric

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hindrance. If this is the case the last C-terminal amino acid does not significantly contribute to the epitope recognition sequence and a potential reduction of the minimal recognition sequence by one amino acid at the C-terminal end has to be considered in the pepspot analysis as used in the present invention.

antibody	position	position
MSR-3 Fab	3-4	18-23
MSR-7 Fab	3-5	19-24
MSR-8 Fab	4-5	18-21
MSR-9 Fab	(1)3-9	18-24
MSR-10 Fab	(4-10)	19-20
MSR-11 Fab	3-7	(18-20)
MSR-26 Fab	3-5	(16)-19-23
MSR-27 Fab	(3)6-9	13-18(20)
MSR-29 Fab		14-16(20)
MSR-37 Fab	(4-6)	(19-24)
MSR-41 Fab	3-7	(17-21)
MSR-42 Fab	(4-9)	(18-24)
MSR 3.4.H7 IgG1	1-3	19-26
MSR 7.9.H2 lgG1	1-4	19-24
MSR 7.9.H7 lgG1	4-6	19-26
MSR 7.2.H2x7.2.L1 lgG1	(1-4) 5-9	18-26
MSR 7.11.H1x7.2.L1 IgG1	4-6	19-26
BAP-2	4-6	
4G8		19-20(23)
BAP-21		32-34
BAP-24		38-40
BAP-1	4-6	
BAP-17		38-40

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Table 2: Pepspot analysis of binding Fabs and full-length IgG antibodies to decapeptides on a cellulose sheet. The numbers refer to the essential amino acids from the A $\beta$ 1-40 sequence which have to be present in the decapeptide for optimal binding of antibody. A weak peptide reactivity, and hence a weak contribution to the epitope, is indicated by brackets.

Example 8: Determination of  $K_D$  values for MS-R Fab and MS-R IgG1 antibody binding to A $\beta$ 1-40 and A $\beta$ 1-42 fibers in *vitro* by surface plasmon resonance (SPR)

Binding of anti-A $\beta$  antibodies (Fabs and IgG1) to fibrillar A $\beta$  was measured online by surface plasmon resonance (SPR), and the affinities of the molecular interactions were determined as described by Johnson, Anal. Biochem. 1991, 198, 268 - 277, and Richalet-Sécordel, Anal. Biochem. 1997, 249, 165 - 173. Biacore 2000 and Biacore3000 instruments were used for these measurements. A $\beta$ 1-40 and A $\beta$ 1-42 fibers were generated in vitro by incubation of synthetic peptides at a concentration of 200 µg/ml in 10 mM Na-acetat buffer (pH 4.0) for three days at 37°C. Electron microscopic analysis confirmed a fibrillar stucture for both peptides, Aβ1-40 showing predominantly shorter (< 1 micron) and A $\beta$ 1-42 predominantly longer (> 1 micron) fibers. These fibers are assumed to represent aggregated Aβ peptides in human AD ~ brain more closely than ill-defined mixtures of amorphous aggregates and unstructured precipitates. The fibers were diluted 1:10 and directly coupled to a "Pioneer Sensor Chip F1" as described in the Instruction Manual of the manufacturer (BIAapplication Handbook, version AB, Biacore AB, Uppsala, 1998). In initial experiments it was found that selected MS-Roche Fabs differed substantially in their reaction kinetics and therefore the mode of data analysis had to be chosen accordingly. For binders with slow kinetics K<sub>D</sub> values were calculated by curve fitting of the time-dependent sensor responses, i. e. from the ratio of k<sub>off</sub>/k<sub>on</sub>. Binders with fast kinetics were analyzed by fitting the concentration-dependent sensor responses at equilibrium (adsorption-isotherms). K<sub>D</sub> values were calculated from the Biacore sensograms based on the total Fab concentration as determined by a protein assay. For the clones derived from the 1st and 2nd affinity maturation cycle the content of

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active Fab in each preparation was determined in the Biacore according to a method described by Christensen, Analytical Biochemistry (1997) 249, 153 –164. Briefly, time-dependent protein binding to A $\beta$ 1-40 fibers immobilized on the Biacore chip was measured during the association phase under mass-limited conditions at different flow rates of the analyte solution. The conditions of mass limitation were realized by immobilizing high amounts of A $\beta$  fibers (2300 response units) on the chip surface of a measuring channel and by working at relatively low analyte concentrations, i. e. 160 nM (based on the total Fab protein concentration).

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A summary of the K<sub>D</sub> values of selected MS-Roche clones identified in the primary screen of the HuCAL library and their corresponding maturated derivatives after the 1<sup>st</sup> and 2<sup>nd</sup> affinity maturation cycle is shown in Table 3. In the 1<sup>st</sup> affinity maturation cycle the heavy chain CDR3 (VH-CDR3) was kept constant and optimization was focussed on diversification of the light chain CDR3 (VL-CDR3). In the 2<sup>nd</sup> affinity cycle diversification of VL-CDR1 and VH-CDR2 was performed. Some of the binders from the 1<sup>st</sup> maturation cycle were converted to full-length human IgG1 antibodies according to the technology developed by MorphoSys as described in Example 6 and K<sub>D</sub> values determined in the Biacore as described above. The K<sub>D</sub> values for full-length IgG1 binding to Aβ1-40 and Aβ1-42 fibers are shown in Table 4.

Matured derivatives from both the L-CDR1 as well as H-CDR2 library after the 2<sup>nd</sup> maturation cycle were identified and allowed combination of light and heavy chains. The cross-cloning strategy is described in Example 13. Either whole light chains, LCDR1 or L-CDR1+2 were exchanged. K<sub>D</sub> values of selected cross-cloned Fabs are shown in Table 8.

Some of the Fabs from the  $1^{st}$  and  $2^{nd}$  maturation cycles and from the cross-cloned binders were converted to full-length human IgG1 antibodies according to the technology developed by MorphoSys as described in Example 6.  $K_D$  values of IgG binding to  $A\beta1$ -40 and  $A\beta1$ -42 fibers were determined in the Biacore. Briefly, a kinetic model for the stepwise formation of a bivalent complex was used, and  $K_D$  values were calculated by Scatchard type analysis of equilibrium binding. Due to the very slow association process at low antibody concentration (several hours to reach equilibrium) equilibrium binding data were obtained by extrapolation of the association curves to long time intervals. The on- and off rates for the formation of

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the monovalent and bivalent complex were determined via the curve fit procedure and used for the extrapolation. Based on these  $R_{eq}$  values a Scatchard analysis was performed and  $K_D$  values for the formation of the monovalent and the bivalent complex were determined. The data are summarized in Table 5. From the curvilinear Scatchard plot a higher (bivalent) and lower (monovalent) affinity interaction was derived for the MS-R IgGs derived from the  $2^{nd}$  affinity maturation cycle and crossclones. These two affinities represent the lower and upper  $K_D$  values of the range indicated in Table 5.

76 MS-R# K<sub>D</sub> Aβ<sub>1-40</sub> K<sub>D</sub> Aβ<sub>1-42</sub> MS-R# K<sub>D</sub> Aβ<sub>1-40</sub> K<sub>D</sub> Aβ<sub>1-42</sub> MS-R# K<sub>D</sub> Aβ<sub>1-40</sub> K<sub>D</sub> Aβ<sub>1-42</sub> Secreted clones from пM nM nΜ nΜ 3 930 1300 7 1100 1714 8 850 1000 primary screen 1 st affinity maturation 58 42 3.2 52 240 7.2 22 24 8.1 24 64 3.3 38 104 23 88 8.2 7.3 3.4 32 103 7.4 28 103 3.6 40 68 7.9 31 93 22 74 7.11 7.12 28 60 2<sup>nd</sup> affinity maturation 3.2H1 4.4 3.3 7.2H1 9.3 10.2 8.1H1 13.6 9.2 8.2H1 2.1<sup>a</sup> 3.2H2 5.2 1.1 7.2H2 8.2 8.2 1.6<sup>a</sup> 3.3H1 19.4 7.2H3 45.4 8.2H3 17.1 5.3 n.d. 3.1 3.3H2 10.6 22.8 7.2H4 5.9 5.0 8.2H4 12.1 11.9 7.2H5 3.3H3 3.3 8.0 10.1 8.2L1 4.8 3.7 1.4 7.2H6 3.4H1 13.5 14.0 1.0 n.d. 7.2H7 3.4H3 6.7 8.4 15.5 8.1 3.4H4 33.0 43.0 7.2H8 1.5 2.1 3.4H5 26.5 36.0 7.2L1 13.3 12.7 7.2L2 3.4H6 49.0 60.0 5.6 4.0 7.2L4 3.4H7 19.2 31.7 1.1 1.1 7.3H1 3.4H8 10.7 26.5 8.0 11.2 7.3L1 4.5 3.4H9 21.7 18.6 6.0 3.4H10 8.1 10.1 7.4H1 8.0 6.6 3.4H11 19.5 8.3 7.4H2 9.9 6.2 3.4H12 25.5 27.0 7.9H1 4.9 5.4 32.3 7.9H2 3.4H13 18.8 5.0 5.7 13.3 7.9H3 4.2 2.8 3.4H14 16.8 3.4H16 25.5 15.6 7.9H4 4.8 4.2 2.0 3.4H17 4.3 7.9H5 1.7 1.8 3.4H18 17.1 10.0 7.9H6 1.2 1.2 9.3 7,9H7 0.9 3.4L7 9.3 1.0 3.4L8 6.2 13.0 7.9H8 0,8 0.7 16.3 7.9H9 0.9 0.9 3.4L9 9.1 5.3 2.6 7.9L1 1.0 3,4L11 1.1 18.9 3.6H1 23.1 7.9L2 1.0 0.5 3.6H2 19.8 54.0 7.11H1 12.7 6.7 3.6H3 5.4 7.5 7.11H2 0.3 0.3 3.6H4 13.0 7.8 7.11H3 6.6 4.4 8.2 3.6H5 6.0 7.11H4 1.0 1.7 36.0 3.6H6 11.8 7.11H5 3.4 1.7 2.5 3.6H8 2.5 7.11L1 1.1 1.2 15.6 7.12H1 0.6 0.8 3.6L1 11.1 3.6L2 13.7 13.1 7.12L1 n.d. 3.8 7.12L2 4.0 5.4 7.12L3 8.0 0.9 7.12L4 2.0 0.6

7.12L5

7.12L6

7.12L7

8.0

n.d.

n.d.

0.6

n.d.

n.d.

Table 3

**Table 3:**  $K_D$  values for MS-R Fab binding to  $A\beta1$ -40 and  $A\beta1$ -42 fibers as determined in the Biacore. For the clones derived from the 1<sup>st</sup> and 2<sup>nd</sup> affinity maturation cycle the values are corrected for the content of active Fab present in each sample as described in the text. <sup>a</sup>, values were calculated from the concentration-dependent sensor responses at equilibrium; n.d., not determined.

Table 4:

MS-R#	$\mathbf{K_{D}} \mathbf{A} \mathbf{\beta}_{1-40}$ $nM$	$\mathbf{K_D} \mathbf{A} \mathbf{\beta_{1-42}}$ $nM$
3.3 lgG1	3.7	6.6
7.11 lgG1 7.12 lgG1	2.3 3.1	5.7 13.7
8.1 laG1	6.6	12.3

**Table 4:**  $K_D$  values for MS-R IgG1 binding to  $A\beta1$ -40 and  $A\beta1$ -42 fibers as determined in the Biacore. The IgGs were derived from MS-R Fabs selected after the 1<sup>st</sup> affinity maturation cycle. The values are corrected for the content of active MS-R IgGs present in each sample as described in the text.

	MS-R IgG1	$K_D A\beta_{1-40}$	$\mathbf{K}_{\mathbf{D}} \mathbf{A} \mathbf{\beta}_{1-42}$
Selected clones from		nM	nM
1 <sup>st</sup> affinity maturation	3.3	3.7	6.6
	7.11	2.3	5.7
	7.12	3.1	13.7
	8.1	6.6	12.3
2 <sup>nd</sup> affinity maturation	3.4.H7	0.10-0.30	0.10-0.30
	7.2.H4	0.09-0.30	0.10-0.66
	7.9.H2	0.12-0.42	0.11-0.38
	7.9.H3	0.10-0.50	0.10-0.40
	7.9. <b>H</b> 7	0.25-0.69	0.24-0.70
	7.12.L1	1.20-3.50	0.74-2.90
	8.2.H2	0.16-1.00	0.12-0.92
cross-cloned Fabs	3.6.H5x3.6.L2	0.20-1.03	0.20-0.95
	3.6.H8x3.6.L2	0.22-0.95	0.22-0.82
	7.4.H2x7.2.L1	0.12-0.63	0.12-0.56
	7.11.H1x7.2.L1	0.14-0.66	0.15-0.67
	7.11.H1x7.11.L1	0.11-0.70	0.13-0.70

**Table 5:**  $K_D$  values for MS-R IgG1 binding to Aβ1-40 and Aβ1-42 fibers as determined in the Biacore. The IgGs were derived from MS-R Fabs selected after the 1<sup>st</sup> and 2<sup>nd</sup> affinity maturation cycle and from crosscloned Fabs. The values are corrected for the content of active MS-R IgGs present in each sample as described in the text. The two  $K_D$  values given for MS-R IgGs derived from the 2<sup>nd</sup> affinity maturation step and cross-cloned binders represent higher and lower affinity interaction as calculated from the curvilinear Scatchard plots. With a number of additional MS-R IgGs (for example MS-R IgG 7.9.H2x7.12.L2 and MS-R IgG 7.9.H4x7.12.L2), complex curvilinear Scatchard blots were obtained and determination of  $K_D$ -values was therefore not possible.

Example 9: Staining of genuine human amyloid plaques in brain sections of an Alzheimer's Disease patient by indirect immunofluorescence

Selected MS-Roche Fabs and full-length IgG1 were tested for binding to β-amyloid plaques by immunohistochemistry analysis. Cryostat sections of unfixed tissue from human temporal cortex (obtained postmortem from a patient that was positively diagnosed for Alzheimer's disease) were labeled by indirect immunofluorescence using MS-Roche Fabs or full-length human IgG1 antibodies at various concentrations. Fabs and IgG1 antibodies were revealed by goat anti-human affinity-purified F(ab')<sub>2</sub> fragment conjugated to Cy3 and goat anti-human (H+L) conjugated to Cy3, respectively. Both secondary reagents were obtained from Jackson Immuno Research. Controls included an unrelated Fab and the secondary antibodies alone, which all gave negative results. Typical examples of plaque stainings with selected MS-Roche Fabs and MS-Roche IgG1 antibodies are shown in Figures 5 to 7.

# Example 10: Polymerization Assay: Prevention of Aβ aggregation

Synthetic  $A\beta$  when incubated in aqueous buffer over several days spontaneously aggregates and forms fibrillar structures which are similar to those seen in amyloid deposits in the brains of Alzheimer's Disease patients. We have developed an *in vitro* assay to measure incorporation of biotinylated  $A\beta$  into preformed  $A\beta$  aggregates in order to analyze the  $A\beta$ -neutralizing potential of anti- $A\beta$  antibodies and other  $A\beta$ -binding proteins such as albumin (Bohrmann et al., 1999, J. Biol. Chem. 274, 15990-15995). The effect of small molecules on  $A\beta$  aggregation can also be analyzed in this assay.

### Experimental procedure:

NUNC Maxisorb microtiter plates (MTP) are coated with a 1:1 mixture of A $\beta$ 1-40 and A $\beta$ 1-42 (2  $\mu$ M each, 100  $\mu$ l per well) at 37°C for three days. Under these conditions highly aggregated, fibrillar A $\beta$  is adsorbed and immobilized on the surface of the well. The coating solution is then removed and the plates are dried at room temperature for 2-4 hours. (The dried plates can be stored at –20°C). Residual binding sites are blocked by adding 300  $\mu$ l/well phosphate-buffered saline containing 0.05 % Tween 20 (T-PBS) and 1 % bovine serum albumin (BSA). After 1-2 hours incubation at room temperature the plates are washed 1 x with 300  $\mu$ l T-PBS. A

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solution of 20 nM biotinylated A $\beta$ 1-40 in 20 mM Tris-HCI, 150 mM NaCl pH 7.2 (TBS) containing 0.05 % NaN $_3$  and serially diluted antibody is added (100  $\mu$ l/well) and the plate incubated at 37°C overnight. After washing 3 x with 300  $\mu$ l T-PBS a streptavidin-POD conjugate (Roche Molecular Biochemicals), diluted 1:1000 in T-PBS containing 1% BSA, is added (100  $\mu$ l/well) and incubated at room temperature for 2 hours. The wells are washed 3 x with T-PBS and 100  $\mu$ l/well of a freshly prepared tetramethyl-benzidine (TMB) solution are added. [Preparation of the TMB solution: 10 ml 30 mM citric acid pH 4.1 (adjusted with KOH) + 0.5 ml TMB (12 mg TMB in 1 ml acetone + 9 ml methanol) + 0.01 ml 35 % H $_2$ O $_2$ ]. The reaction is stopped by adding 100  $\mu$ l/well 1 N H $_2$ SO $_4$  and absorbance is read at 450 nm in a microtiter plate reader.

### Result:

Figure 8 shows that MS-Roche IgG1 antibodies prevented inorporation of biotinylated A\u00e41-40 into preformed A\u00e41-40/A\u00e41-42 aggregates. The A\u00e4-neutralizing capacity of these full-length human IgGs was similar to that of the mouse monoclonal antibody BAP-1 which had been generated by a standard immunization procedure and specifically recognizes amino acid residues 4-6 of the Aßpeptide when analyzed by the Pepspot technique as described in example 7. Mouse monoclonal antibody BAP-2 which also reacts exclusively with amino acids 4-6 (Brockhaus, unpublished) was significantly less active in this assay. An even lower activity was found with the Aβ1-40 C-terminal specific antibody BAP-17 (Brockhaus, Neuroreport 9 (1998), 1481-1486) and the monoclonal antibody 4G8 which recognizes an epitope between position 17 and 24 in the Aß sequence (Kim, 1988, Neuroscience Research Communication Vol. 2, 121-130). BSA at a concentration of up to 10 μg/ml did not affect incorporation of biotinylated Aβ and served as a negative control. However, at higher concentrations, i. e. > 100 μg/ml, BSA has been reported to inhibit binding of biotinylated AB into preformed AB fibers (Bohrmann, (1999) J Biol Chem 274 (23), 15990-5) indicating that the interaction of BSA with Aβ is not of high affinity.

# Example 11: De-polymerization Assay: Release of biotinylated A $\beta$ from aggregated A $\beta$

In a similar experimental setup we have tested the potential of MS-Roche IgG antibodies to induce depolymerization of aggregated A $\beta$ . Biotinylated A $\beta$ 1-40 was first incorporated into preformed A $\beta$ 1-40/A $\beta$ 1-42 fibers before treatment with various anti-A $\beta$  antibodies. Liberation of biotinylated A $\beta$  was measured using the same assay as described in the polymerization assay.

#### Experimental procedure:

NUNC Maxisorb microtiter plates (MTP) are coated with a 1:1 mixture of A $\beta$ 1-40 and A $\beta$ 1-42 as described in the polymerization assay. For incorporation of biotinylated A $\beta$  the coated plates are incubated with 200  $\mu$ l/well 20 nM biotinylated A $\beta$ 1-40 in TBS containing 0.05 % NaN<sub>3</sub> at 37°C overnight. After washing the plate with 3 x 300  $\mu$ l/well T-PBS, antibodies serially diluted in TBS containing 0.05 % NaN<sub>3</sub> were added and incubated at 37°C for 3 hours. The plate was washed and analyzed for the presence of biotinylated A $\beta$ 1-40 as described above.

#### Result:

Figures 9A to D shows that the inventive antibodies induced de-polymerization of aggregated A $\beta$  as measured by the release of incorporated biotinylated A $\beta$ 1-40. The MS-R antibodies and the mouse monoclonal antibody BAP-1 were similarly active whereas the BAP-2, BAP-17 and 4G8 antibodies were clearly less efficient in liberating biotinylated A $\beta$  from the bulk of immobilized A $\beta$  aggregates. BAP-1 can clearly be differentiated from the MS-R antibodies by its reactivity with cell surface full-length APP (see Figure 15), and antibodies like BAP-1 with such properties are not useful for therapeutic applications as potential autoimmunological reactions may be induced. It is interesting to note that BAP-2, despite its specificity for amino acid residue 4-6 which is exposed in aggregated A $\beta$  has a clearly lower activity in this assay indicating that not all N-terminus specific antibodies a priori are equally efficient in releasing A $\beta$  from preformed aggregates. The MS-Roche IgGs are clearly superior to BAP-2 with respect to the depolymerizing activity. The relatively low

efficiency of BAP-17 (C-terminus-specific) and 4G8 (amino acid residues 16-24-specific) in this assay is due to the cryptic nature of these two epitopes in aggregated A $\beta$ . As already noted in the polymerization assay, BSA at the concentrations used here had no effect on aggregated A $\beta$ .

The MS-R antibodies derived from the 2<sup>nd</sup> affinity maturation cycle and from the cross-cloned binders show in general a higher efficacy in the de-polymerization assay (comparison of figure 9A with figures 9B and C), which is consistent with the increased binding affinity of these antibodies (see tables 3-5). The monoclonal antibodies AMY-33 and 6F/3D have been reported to prevent Aβ aggregation in vitro under certain experimental conditions (Solomon, (1996) Proc. Natl. Acad. Sci. USA 93, 452-455; AMY-33 and 6F/3D antibodies were obtained from Zymed Laboratories Inc., San Francisco (Order No. 13-0100) and Dako Diagnostics AG, Zug, Switzerland (Order No. M087201), respectively). As demonstrated in figure 9D both of these antibodies were completely inactive in the de-polymerization assay.

## **EXAMPLE 12: Epitope analysis by ELISA on peptide conjugates.**

The following heptapeptides (single letter code) were obtained by solid-phase synthesis and purified by liquid chromatography using the techniques known in the art.

**AEFRHDC** 

**EFRHDSC** 

**FRHDSGC** 

**RHDSGYC** 

**HDSGYEC** 

**DSGYEVC** 

**SGYEVHC** 

YEVHHQC

**EVHHQKC** 

VHHQKLC

**HHQKLVC** 

**HQKLVFC** 

**QKLVFFC** 

**KLVFFAC** 

LVFFAEC

VFFAEDC

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FFAEDVC
FAEDVGC
AEDVGSC
EDVGSNC
DVGSNKC
VGSNKGC
GSNKGAC
CSNKGAI
CNKGAII
CKGAIIG
CGLMVGG
CMVGGVV

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The peptides were dissolved in DMSO to arrive at 10 mM concentration.

Bovine Albumin (essentially fatty acid free BSA, Sigma Lot 112F-9390) was dissolved to 10 mg/ml in 0.1M sodium bicarbonate and activated by addition per ml of 50 µl of a 26 mg/ml solution of N-succinmidyl-maleinimido propionate (NSMP, Pierce) in DMSO. After 15 minutes reaction at room temperature the activated BSA was purified by gel filtration (NAP-10, Pharmacia) in PBS with 0.1% sodium azide as solvent. 50 µl of NSMP activated BSA (6.7 mg/ml) was diluted with 50 µl of PBS, 0.1% sodium azide and 10 µl of peptide solution (1 mM in DMSO) was added. As negative control activated BSA was mock-treated without peptide addition. After 4 hrs at room temperature the reaction was stopped by addition of 10 µl of 10mM Cystein. An aliquot of the conjugate reaction mixture was diluted 1:100 with 0.1M sodium bicarbonate buffer and immediately filled into the wells (100 µl) of ELISA plates (Nunc Immuno-Plate). After standing 16 hrs at 4°C 100 µl blocking buffer (as above) was added to each well and incubated for another 30 minutes. The plates were washed with 2x300 µl/well TBST (as above) and filled with 100 µl antibody at 10 μg/ml or 2 μg/ml in blocking buffer. The plates were kept 16 hours at 4°C and washed with 2x300 µl TBST. 100 µl/well HRP-conjugated anti-human lg H+L (Pierce, dilution 1:1000 with blocking buffer) was added and incubated for 1 hour at ambient temperature. The plates were washed with 3x300ul/well TBST. Colour development was started by addition of 100 µl tetra-methyl benzidine/hydrogen peroxide reagent. The reaction was stopped after 5 minutes by addition of 100 µl/well 1M sulfuric acid and the optical density is measured by an opticalreader (Microplate Reader 3550, BioRad) at 450 nm. For comparison mouse monoclonal

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antibodies were analysed in the same way, except using as revealing agent HRP-labelled anti-mouse Ig instead of anti-human Ig.

Employing specific of the above described heptapeptides derived from A $\beta$ , specific ELISA-tests as described herein above were carried out. Preferably, inventive antibodies comprise antibodies which show, as measured by of optical densities, a signal to background ratio above "10" when their reactivity with an A-beta derived peptide (AEFRHD; amino acid 2 to 7 of A-beta) is compared to an non-related protein/peptide like BSA. Most preferably, the ratio of optical densities is above "5" for a corresponding reaction with at least one of the following three A $\beta$  derived peptides: (VFFAED; amino acid 18 to 23 of A $\beta$ ) or (FFAEDV; amino acid 19 to 24 of A $\beta$ ) or (LVFFAE; amino acid 17 to 22 of A $\beta$ ).

Corresponding results for the inventive parental and/or maturated antibodies are shown in the following two tables:

Peptide-ration	19-24/2-7	0.17	0.89	0.26	0.26	0.17	0.29	0.24	0.18	0.27	0.72		0.77	0.35	90.0	0.07	90.0	0.50	0.14	0.21	0.39	0.16	0.28	0.22
Peptide-ratio	18-23/2-7	0.29	1.04	0.47	0.44	0.36	0.46	0.41	0.21	0.37	0.88	,	0.88	0.52	0.16	0.30	90'0	0.77	0.46	0.25	0.43	0.65	0.16	0.57
Peptide-ratio	17-22/2-7	0.17	0.36	0.35	0.32	0.28	0.32	0.30	0.16	0.03	0.13	:	0.46	0.17	90'0	0.07	69.0	0.41	0.18	0.08	0.18	0.19	0.04	0.13
Peptide 19-24 Peptide-ratio Peptide-ration	19-24/BSA	4	25	6	6	9	8	6	7	8	23		20	∞	2	2	-	11	4	5	11	5	7	5
1	18-23/BSA	7	29	16	15	13	13	15	<b>∞</b>	11	28	Ć	23	12	5	∞		17	. 13	9	12	20	4	13
Peptide 17-22 Peptide 18-23	17-22/BSA	4	10	12	11	10	6	11	9	₩	4	,	12	4	7	2	11	6	5	7	5	9	_	3
Peptide2-7	2-7/BSA	24	28	34	34	36	28	37	38	30	32	ò	70	23	31	27	16	22	28	24	28	31	25	23
MS-R#		7	∞	7.2	7.3	7.4	6.7	7.11	7.12	8.1	8.2	2.2170	3.2H2	3.3H1	3.3H3	3.4H1	3.4H2	3.4H3	3.4H5	3.4H7	3.4H17	3.4L11	3.6H6	3.6H1

0.16	0.24	0.63	0.55	0.52	0.50	0.45	0.51	0.46	0.19	0.23	0.27	0.22	0.25	0.41	0.59	0.86	0.94	1.22
0.42	0.29	0.63	0.61	0.57	09.0	89.0	0.65	0.56	0.36	0.16	0.57	0.49	0.29	0.36	0.70	0.88	1.05	1.26
0.11	0.21	0.63	0.52	0.52	0.43	0.57	0.52	0.47	0.23	0.08	0.31	0.32	0.25	0.29	09.0	0.77	0.34	09.0
3	6	10	18	12	15	11	16	16	9	8	6	7	8	14	10	25	20	31
8		10	20	13	18	16	20	20	12	9	20	15	6	12	12	25	23	32
2	8	10	17	12	13	14	16	17	7	3	11	10	8	10	10	22	7	15
19	38	16	33	23	30	24	31	36	32	35	35	30	31	34	16	29	22	76
3.6H2	7.2H1	7.2H2	7.2H3	7.2H4	7.2H5	7.2L1	7.4H1	7.4H2	7.9H1	7.9H2	7.9H3	7.9H4	7.11H1	7.11H2	7.12L1	8.1H1	8.2H1	8.2L1

Table 6: Reactivity of MS-R Fabs with BSA-conjugated Abeta heptapeptides 2-7 (AEFRHD), 17-22 (LVFFAE), 18-23 (VFFAED) and 19-24 (FFAEDV). The ratios of the ELISA read-out (optical density) obtained with peptide-conjugated and non-conjugated BSA are given. The signal intensities obtained with the 17-22, 18-23 and 19-24 peptides in relation to the 2-7 peptide are also indicated.

MS-R IgG	AEFRHD	LVFFAE	VFFAED	FFAEDV	Peptide-ratio	Peptide- ratio	Peptide- ratio
#	2-7/BSA	17-22/BSA	18-23/BSA	19-24/BSA	17-22/2-7	18-23/2-7	19-24/2-7
3.3	17	11	16	11	0.65	0.94	0.65
7.12	19	11	13	11	0.58	0.68	0.58
8.1	16	7	16	14	0.44	1.00	0.38
3.4H7	22	3	16	15	0.14	0.73	0.68
7.9H2	13	5	8	6	0.38	0.73	0.46
7.9H3	13	6	8	6	0.46	0.62	0.46
7.9.H7	30	5	16	10	0.17	0.53	0.33
7.11H2	10	6	7	6	0.60	0.70	0.60
8.2.H2	18	10	15	14	0.56	0.83	0.78
3.6.H5x3.6.L2	11	7	9	8	0.64	0.82	0.73
7.11.H2x7.9.L	14	8	10	9	0.57	0.71	0.64
1 (L1) 8.2.H2x8.2.L1	13	20	25	25	1.54	1.92	1.92
Mouse mab			٠				,
BAP-1	21	1	1	1	0.05	0.05	0.05
BAP-2	21	1	1	1	0.05	0.05	0.05
4G8	1	23	20	1	23	20	1
6E10	18	1	1	1	0.06	0.06	0.06
6F/3D*	1	. 1	1	1	1	1	1
Amy 33	16	2	1	3	0.13	0.06	0.19

**Table 7**: Reactivity of MS-R IgGs and mouse monoclonal antibodies BAP-1, BAP-2, 4G8, 6E10 Amy-33 and 6F/3D with BSA-conjugated Aβ heptapeptides 2-7 (AEFRHD), 17-22 (LVFFAE), 18-23 (VFFAED) and 19-24 (FFAEDV). The ratios of the ELISA read-out (optical density) obtained with peptide-conjugated and non-conjugated BSA are given. The signal intensities obtained with the 17-22, 18-23 and 19-24 peptides in relation to the 2-7 peptide are also indicated. \* this antibody is specific for sequence 8-17 and does not recognize N-terminal or middle epitope sequences.

# EXAMPLE 13: Combination of optimized H-CDR2 and L-CDR1 by cross-cloning

The modular design of the HuCAL library allows exchange of complementarity determining regions (CDRs) of two different Fab encoding genes in a simple cloning

step. For a further improvement of affinity the independently optimized H-CDR2 and L-CDR1 from matured clones with the same H-CDR3 were combined, because there was a high probability that this combination would lead to a further gain of affinity (Yang et al., 1995, J.Mol.Biol. 254, 392-403; Schier et al., 1996b, J.Mol.Biol. 263, 551-567; Chen et al., 1999, J.Mol.Biol. 293, 865-881). Whole light chains, or fragments thereof, were transferred from an L-CDR1 optimized donor clone to a H-CDR2 optimized recipient clone. Donor and recipient clones were only combined, if both carried identical H-CDR3 sequences. All donor and recipient clones carried the VH3-Vκ3 framework.

This was accomplished by transferring whole light chains from the L-CDR1-optimized donor clone to the H-CDR2-optimized recipient clone. Epitope specificity was conserved by only combining clones with the same H-CDR3. By light chain exchange a H-CDR2-optimized clone obtained only an optimized L-CDR1, if the exchange occured between clones with the same L-CDR3. If the L-CDR3 of the clones to be combined was different, the H-CDR2-optimized clone acquired in addition to the optimized L-CDR1 another L-CDR3 (L-CDR2 remained the HuCAL consensus sequence (Knappik et al., 2000)) and when derivatives of MS-Roche #7.12 were used as donors of the light chain L-CDR1, 2 and 3 were exchanged in the H-CDR2-optimized acceptor clone. Three different cloning strategies were employed:

- 1) Using restriction endonucleases XbaI and SphI the whole antibody light chain fragment was excised from plasmid 1 (e.g. pMx9\_Fab\_MS-Roche#7.11.H1\_FS) and the thereby obtained vector backbone was then ligated to the light chain fragment of plasmid 2 (e.g. pMx9\_Fab\_MS-Roche#7.2.L1\_FS) generated by XbaI and SphI digest. Thereby a new plasmid (nomenclature: pMx9\_Fab\_MS-Roche#7.11.H1x7.2.L1\_FS) was created encoding L-CDR1,2,3 of parental clone #7.2.L1 and H-CDR1,2,3 of parental clone #7.11.H1.
- 2) Using restriction endonucleases XbaI and Acc65I an L-CDR1 coding fragment was excised from plasmid 1 (e.g. pMx9\_Fab\_MS-Roche#7.11.H2\_FS) and the thereby obtained vector backbone was then ligated to the L-CDR1 fragment of plasmid 2 (e.g. pMx9\_Fab\_MS-Roche#7.12.L1\_FS) generated by XbaI and Acc65I. Thereby a new plasmid (nomenclature: pMx9\_Fab\_MS-

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Roche#7.11.H2x7.12.L1(L-CDR1)\_FS) was created encoding L-CDR1 of parental clone #7.12.L1 while L-CDR2,3 and H-CDR1,2,3 are derived from parental clone #7.11.H2.

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3) Using restriction endonucleases XbaI and BamHI an L-CDR1 and L-CDR2 coding fragment was excised from plasmid 1 (e.g. pMx9\_Fab\_MS-Roche#7.11.H2\_FS) and the thereby obtained vector backbone was then ligated to the L-CDR1 and L-CDR2 fragment of plasmid 2 (e.g. pMx9\_Fab\_MS-Roche#7.12.L1\_FS) generated by XbaI and BamHI digest. Thereby a new plasmid (nomenclature: pMx9\_Fab\_MS-Roche#7.11.H2x7.12.L1(L-CDR1+2)\_FS) was created encoding L-CDR1 and L-CDR2 of parental clone #7.12.L1 while L-CDR3 and H-CDR1,2,3 are derived from parental clone #7.11.H2.

Illustrative examples for the different cloning strategies as well as for sequences donor and recipient clones are given in table 8.

After large scale expression and purification their affinities were determined on A $\beta$  (1-40) fibers. Furthermore,  $K_D$  values for selected cross-cloned MS-R Fab/antibodies are given in appended Table 9.

Binder name	L-CDR1	pos.49	L-CDR2	pos. 85	L-CDR3	H-CDR1	pos.47	H-CDR2	H-CDR3
		1					-		
cloning strategy 1)	<b>.</b>				▶.				
MS-Roche #7.11.H1	RASQSVSSSYLA	>-	GASSRAT	<b>-</b>	QQVYSPPH	GFTFSSYAMS	>	GINAAGFRTYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.2.L1	RASQYVDRTYLA	>	GASSRAT	H	QQIYSFPH	GFTFSSYAMS	>	AISGSGGSTYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.11.H1x7.2.L1	RASQYVDRTYLA	٨	GASSRAT	Т	QQІҮЅҒРН	GFTFSSYAMS	W	GINAAGFRTYYADSVKG	GKGNTHKPYGYVRYFDV
cloning strategy 2)	<b>&gt;</b>								
MS-Roche #7.11.H2	RASQSVSSSYLA	>	GASSRAT	F	ООЛУЗРРН	GFTFSSYAMS	*	AINANGYKKYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.12.L1	RASQYVFRRYLA	S	GSSNRAT	>	LQLYNIPN	GFTFSSYGMS	*	NISGSGSSTYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.11.H2x7.12.L1(LCDR1)	RASQYVFRRYLA	>	GASSRAT	Ь	QQVYSPPH	GFTFSSYAMS	М	AINANGYKKYYADSVKG	GKGNTHKPYGYVRYFDV
cloning strategy 3)			_						
MS-Roche #7.11.H2	RASQSVSSSYLA	>	GASSRAT	F	ООЛУЗРРН	GFTFSSYAMS	×	AINANGYKKYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.12.L1	RASQYVFRRYLA	S	GSSNRAT	>	LQLYNIPN	GFTFSSYGMS	×	NISGSGSSTYYADSVKG	GKGNTHKPYGYVRYFDV

MS-Roche #7.11.H2x7.12.L1(LCDR1+2)	RASQYVFRRYLA	S	GSSNRAT	F	Надуууд	GFTFSSYAMS	*	AINANGYKKYYADSVKG	GKGNTHKPYGYVRYFDV
Binder name	L-CDR1	pos.49	L-CDR2	pos. 85	L-CDR3	H-CDR1	pos.47	H-CDR2	H-CDR3
MS-Roche #3.6H5 MS-Roche #3.6L2	RASQSVSSSYLA RASOFLSRYYLA	> >	GASSRAT	>>	QQTYNYPP	GFTFSSYAMS GFTFSSYAMS	3 3	AISESGKTKYYADSVKG AISGSGGSTYYADSVKG	LTHYARYYRYFDV LTHYARYYRYFDV
MS-Roche #3.6H5x3.6L2	RASQFLSRYYLA	>	GASSRAT	>	QQTYNYPP	GFTFSSYAMS	>	AISESGKTKYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.6H8	RASOSVSSSYLA	>-	GASSRAT	>	OOTYNYPP	GFTFSSYAMS	*	AISEYSKFKYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.6L2 MS-Roche #3.6H8x3.6L2	RASQFLSRYYLA RASQFLSRYYLA	> >	GASSRAT GASSRAT	>>	QQTYNYPP QQTYNYPP	GFTFSSYAMS GFTFSSYAMS	<b>≥</b> ≥	AISGSGGSTYYADSVKG AISEYSKFKYYADSVKG	LTHYARYYRYFDV LTHYARYYRYFDV
	-	;	100				,	OW DANKER AND	CICANTLINDVCW/DVEDW
MS-Kocne #7.4.H2	KASŲSVSSSYLA	>	GASSKAI	>	ŲŲIYNFPH	GFIFSSYAMS	A	AINTINGAKIT TADSVNG	GRGWITINFI GIVALI DA
MS-Roche #7.2.L1	RASQYVDRTYLA	>	GASSRAT	F	QQIYSFPH	GFTFSSYAMS	>	AISGSGGSTYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.4.H2x7.2.L1	RASQYVDRTYLA	>	GASSRAT	Н	QQIYSFPH	GFTFSSYAMS	8	AINYNGARIYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7,9H2	RASQSVSSSYLA	>	GASSRAT	<b>-</b>	LQIYNMPI	GFTFSSYAMS	Μ	AINADGNRKYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.12L2	RASQRFFYKYLA	S	GSSNRAT	>	LQLYNIPN	GFTFSSYGMS	>	NISGSGSSTYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.9H2x7.12L2	RASQRFFYKYLA	s	GSSNRAT	>	LQLYNIPN	GFTFSSYAMS	M	AINADGNRKYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.9H4	RASQSVSSSYLA	>	GASSRAT	⊢	LQIYNMPI	GFTFSSYAMS	×	AINAVGMKKFYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.12.L2	RASQRFFYKYLA	S	GSSNRAT	>	LQLYNIPN	GFTFSSYGMS	×	NISGSGSSTYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.9H4x7.12L2	RASQRFFYKYLA	S	GSSNRAT	>	LQLYNIPN	GFTFSSYAMS	M	AINAVGMKKFYADSVKG	GKGNTHKPYGYVRYFDV

7.0									
MS-Roche #7.11H1	RASQSVSSSYLA	>-	GASSRAT	Τ	Нач	GFTFSSYAMS	W	GINAAGFRTYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.11L1	RASQRILRIYLA	Y	GASSRAT	T	Начизууд	GFTFSSYAMS	M	AISGSGGSTYYADSVKG	GKGNTHKPYGYVRYFDV
Binder name	L-CDR1	pos.49	L-CDR2	pos. 85	L-CDR3	H-CDR1	pos.47	H-CDR2	H-CDR3
MS-Roche #7.11H1x7.11L1	RASQRILRIYLA	γ	GASSRAT	⊢	Нач	GFTFSSYAMS	*	GINAAGFRTYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.11H1	RASQSVSSSYLA	>-	GASSRAT	⊢	ООЛУЅРРН	GFTFSSYAMS	≥	GINAAGFRTYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.2L1	RASQYVDRTYLA	Υ	GASSRAT	<b>—</b>	QQIYSFPH	GFTFSSYAMS	≱	AISGSGGSTYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.11H1x7.2L1	RASQYVDRTYLA	λ	GASSRAT	L	ООІҮЅЕРН	GFTFSSYAMS	×	GINAAGFRTYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #3.3H1	RASQSVSSSYLA	Y	GASSRAT	>	HOMSSYPP	GFTFSSYAMS	М	VISEKSRFIYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4L9	RASRRIHVYYLA	Υ	GASSRAT	⊢	QQTYDYPP	GFTFSSYAMS	×	AISGSGGSTYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.3H1x3.4L9	RASRRIHWYYLA	>	GASSRAT	Н	ООТУДУРР	GFTFSSYAMS	×	VISEKSRFIYYADSVKG	LTHYARYYRYFDV
MS-Roche #3 4H1	DASOCIVECCVI A	>	CACCDAT	۱	gavavtoo	CETECCVAMC	A	ATCETCIBIONAPONIC	I THYABVVBVED!/
MS-Roche #3.4L9	RASRRIHVYYLA	>-	GASSRAT	-  -	OOTYDYPP	GETESSYAMS	≥ ≥	AISGSGGSTYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4H1x3.4L9	RASRRIHVYYLA	>	GASSRAT	⊥	ООТУДУРР	GFTFSSYAMS		AISETSIRKYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4H3	RASQSVSSSYLA	Y	GASSRAT	T	ddydytog	GFTFSSYAMS	×	VISQTGRKIYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4L7	RASQRLGRLYLA	<b>\</b>	GASSRAT	Т	QQTYDYPP	GFTFSSYAMS	W	AISGSGGSTYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4H3x3.4L7	RASQRLGRLYLA	>	GASSRAT	Н	QQTYDYPP	GFTFSSYAMS	8	VISQTGRKTYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4H3	RASQSVSSSYLA	>	GASSRAT	F	OOTYDYPP	GFTFSSYAMS	8	VISOTGRKIYYADSVKG	LTHYARYYRYFDV
MS-Roche #3,4L9	RASRRIHVYYLA	Υ	GASSRAT	Τ	QQTYDYPP	GFTFSSYAMS	×	AISGSGGSTYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4H3x3.4L9	RASRRIHVYYLA	>	GASSRAT	⊢	dd.AQALÒÒ	GFTFSSYAMS	Μ	VISQTGRKIYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4H7	RASQSVSSSYLA	>	GASSRAT	F	ООТУБУРР	GFTFSSYAMS	×	VISETGKNIYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4L9	RASRRIHVYYLA	>	GASSRAT	⊢	QQTYDYPP	GFTFSSYAMS	≥	AISGSGGSTYYADSVKG	LTHYARYYRYFDV
MS-Roche #3.4H7x3.4L9	RASRRIHVYYLA	>	GASSRAT	Τ	QQTYDYPP	GFTFSSYAMS	W	VISETGKNIYYADSVKG	LTHYARYYRYFDV

-	П			$\top$	Т	$\overline{\Box}$		1		_				Т				1			П	$\neg$	
LTHYARYYRYFDV	LTHYARYYRYFDV	H-CDR3	LTHYARYYRYFDV	LTHYARYYRYFDV	LTHYARYYRYFDV	LTHYARYYRYFDV	GKGNTHKPYGYVRYFDV	GKGNTHKPYGYVRYFDV	GKGNTHKPYGYVRYFDV		GKGNTHKPYGYVRYFDV	GKGNTHKPYGYVRYFDV	GKGNTHKPYGYVRYFDV		GKGNTHKPYGYVRYFDV	GKGNTHKPYGYVRYFDV	GKGNTHKPYGYVRYFDV		GKGNTHKPYGYVRYFDV	GKGNTHKPYGYVRYFDV	GKGNTHKPYGYVRYFDV		GKGNTHKPYGYVRYFDV
VISETGKNIYYADSVKG	AISGSGGSTYYADSVKG	H-CDR2	VISETGKNIYYADSVKG	AISESGKTKYYADSVKG	AISGSGGSTYYADSVKG	AISESGKTKYYADSVKG	AINGTGMKKYYADSVKG	AISGSGGSTYYADSVKG	AINGTGMKKYYADSVKG		AINYNGARIYYADSVKG	NISGSGSSTYYADSVKG	AINYNGARIYYADSVKG		AINADGNRKYYADSVKG	AISGSGGSTYYADSVKG	AINADGNRKYYADSVKG		AINANGYKKYYADSVKG	AISGSGGSTYYADSVKG	AINANGYKKYYADSVKG		AINADGNRKYYADSVKG
8	M	pos.47	8	>	≥	≥	≯	8	Μ		M	*	≥		×	M	3		≥	*	≥		≯
GFTFSSYAMS	GFTFSSYAMS	H-CDR1	GFTFSSYAMS	GFTFSSYAMS	GFTFSSYAMS	GFTFSSYAMS	GFTFSSYAMS	GFTFSSYAMS	GFTFSSYAMS		GFTFSSYAMS	GFTFSSYGMS	GFTFSSYAMS		GFTFSSYAMS	GFTFSSYAMS	GFTFSSYAMS		GFTFSSYAMS	GFTFSSYAMS	GFTFSSYAMS	diameter of the state of the st	GFTFSSYAMS
ООТУВУРР	ООТУБУРР	L-CDR3	QQTYDYPP	OOTYNYPP	QQTYNYPP	QQTYNYPP	QQIYSFPH	QQIYSFPH	QQIYSFPH		QQIYNFPH	LQLYNIPN	LQLYNIPN		LQIYNMPI	QQIYSFPH	QQIYSFPH		H44SVVQQ	НЬЫХІДОО	QQIYSFPH		LQIYNMPI
<u>,</u> ⊢	-	pos. 85	⊢	>	>	>	<b>⊢</b>	<b>-</b>	Н		^	>	>		⊢	⊢	⊢		<b>—</b>	1	$\vdash$		⊢
GASSRAT	GASSRAT	L-CDR2	GASSRAT	GASSRAT	GASSRAT	GASSRAT	GASSRAT	GASSRAT	GASSRAT		GASSRAT	GSSNRAT	GSSNRAT		GASSRAT	GASSRAT	GASSRAT		GASSRAT	GASSRAT	GASSRAT		GASSRAT
>	<b>X</b>	pos.49	>	>	>	<b>&gt;</b>	Y	>	<b>&gt;</b>		٨	S	S		<b>&gt;</b>	>-	>		>-	Y	>		>
RASQSVSSSYLA	RASQRLGRLYLA	L-CDR1	RASQRLGRLYLA	RASQSVSSSYLA	RASQFIQRFYLA	RASQFIQRFYLA	RASQSVSSSYLA	RASQYVDRTYLA	RASQYVDRTYLA		RASQSVSSSYLA	RASQRFFYKYLA	RASQRFFYKYLA		RASQSVSSSYLA	RASQYVDRTYLA	RASQYVDRTYLA		RASQSVSSSYLA	RASQYVDRTYLA	RASQYVDRTYLA		RASQSVSSSYLA
MS-Roche #3.4H7	MS-Roche #3.4L7	Binder name	MS-Roche #3.4H7x3.4L7	MS-Roche #3.6H5	MS-Roche #3.6L1	MS-Roche #3.6H5x3.6L1	MS-Roche #7.2H2	MS-Roche #7,2L1	MS-Roche #7.2H2x7.2L1		MS-Roche #7,4H2	MS-Roche #7.12L2	MS-Roche #7,4H2x7.12L2		MS-Roche #7,9H2	MS-Roche #7.2L1	MS-Roche #7.9H2x7,2L1		MS-Roche #7.11H2	MS-Roche #7.2L1	MS-Roche #7.11H2x7.2L1		MS-Roche #7.9H2

MS-Roche #7.12 <u>1.1</u>	RASQYVFRRYLA	S	GSSNRAT	>	LQLYNIPN	GFTFSSYGMS	*	NISGSGSSTYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.9H2x7.12L1	RASQYVFRRYLA	S	GSSNRAT	>	LQLYNIPN	GFTFSSYAMS	×	AINADGNRKYYADSVKG	GKGNTHKPYGYVRYFDV
Binder name	L-CDR1	pos.49	L-CDR2	pos. 85	L-CDR3	H-CDR1	pos.47	H-CDR2	H-CDR3
MS-Roche #7.11H2	RASQSVSSSYLA	γ	GASSRAT	L	ООЛУЗРРН	GFTFSSYAMS	*	AINANGYKKYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.9L1	RASQRLSPRYLA	>	GASSRAT	1	TOINNMI	GFTFSSYAMS	×	AISGSGGSTYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.11H2x7.9L1 RASQRLSPRYLA	RASQRLSPRYLA	>	GASSRAT	⊢	LQIYNMPI	GFTFSSYAMS	≥	AINANGYKKYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #8,1H1	RASQSVSSSYLA	>	GASSRAT	L	QQLSNYPP	GFTFSSYAMS	8	AISRSGSNIYYADSVKG	LLSRGYNGYYHKFDV
MS-Roche #8.2L1	RASQRVSGRYLA	Υ	GASSRAT	⊢	QQLSSYPP	GFTFSSYAMS	≥	AISGSGGSTYYADSVKG	LLSRGYNGYYHKFDV
MS-Roche #8.1H1x8.2L1	RASQRVSGRYLA	>	GASSRAT	⊢	QQLSSYPP	QQLSSYPP GFTFSSYAMS	Μ	AISRSGSNIYYADSVKG	LLSRGYNGYYHKFDV
MS-Roche #7.11H2	RASQSVSSSYLA	>	GASSRAT	<b> </b>	QQVYSPPH	GFTFSSYAMS	≱	AINANGYKKYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.12L1	RASQYVFRRYLA	S	GSSNRAT	^	LQLYNIPN	GFTFSSYGMS	*	NISGSGSSTYYADSVKG	GKGNTHKPYGYVRYFDV
MS-Roche #7.11H2x7.12L1	RASQYVFRRYLA	S	GSSNRAT	۸	LQLYNIPN	GFTFSSYAMS	8	AINANGYKKYYADSVKG	GKGNTHKPYGYVRYFDV

Table 8 Arrows indicate the location of restriction enzyme sites used to digest corresponding plasmids

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MS-R#	K <sub>D</sub> Aβ <sub>1-40</sub>	K <sub>D</sub> Aβ <sub>1-42</sub>
	nΜ	nM
	0.40	
3.3H1x3.4L9	2.16	2.97
3.4H1x3.4L9	0.25	0.5
3.4H3x3.4L7	0.92	0.92
3.4H3x3.4L9	1.05	0.93
3,4H7x3.4L9	2.66	3.51
3.4H7x3.4L7	1.19	1.23
3.6H5x3.6L1	1.25	1.04
3.6H5x3.6L2	1.26	0.84
7.2H2x7.2L1	1.29	1.43
7.4H2x7.2L1	1.4	1.4
7.4H2x7.12L2	1.4	1.8
7.9H2x7.2L1(L1)	1.4	1.4
7.9H2x7.12L1	1.2	1.1
7.9H2x7.12L2(L1+2)	0.4	0.4
7.11H1x7.2L1	1.75	1.39
7.11H1x7.11L1	0.41	0.47
7.11H2x7.2L1(L1)	1	0.6
7.11H2x7.9L1 (L1)	0.1	1
8.1H1x8.2L1	1.3	1.6

**Table 9:**  $K_D$  values for crosscloned MS-R Fab binding to Aβ1-40 and Aβ1-42 fibers as determined in the Biacore. The preparation of crosscloned Fabs is described in example 13. The  $K_D$  values were determined by kinetic curve fittings and corrected for the content of active Fab present in each sample as described in the text. Some of the Fabs were additionally purified by size exclusion chromatography or preparative ultracentrifugation to remove aggregated material. (L1), the H-CDR2-matured acceptor clone received only L-CDR1 from the L-CDR1 improved donor clone; (L1+2), the H-CDR2-matured acceptor clone received L-CDR1+2 from the L-CDR1 improved donor clone.

Example 14: In vivo amyloid plaque decoration in a mouse model of Alzheimer's disease as revealed by confocal laser scanning microscopy and colocalization analysis.

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Selected MS-R IgG1 antibodies were tested in APP/PS2 double transgenic mice (Reference: Richards et al., Soc. Neurosci. Abstr., Vol. 27, Program No. 5467, 2001) for amyloid plaque decoration in vivo. The antibodies (1 mg/mouse) were administered i.v. and after 3 days the brains were perfused with saline and prepared for cryosection. In another study the mice were exposed to higher concentrations of the antibodies, i.e. 2 mg injected i.v. at day 0, 3, and 6, and sacrificed at day nine. The presence of the antibodies bound to amyloid plaques was assessed on unfixed cryostat sections by double-labeled indirect immunofluorescence using goat antihuman IgG (H+L) conjugated to either Cy3 (#109-165-003, Jackson Immuno Research) followed by BAP-2-Alexa488 immunoconjugate. Imaging was done by confocal laser microscopy and image processing for quantitative detection of colocalizations by IMARIS and COLOCALIZATION software (Bitplane, Switzerland). Typical examples are shown in Figures 10-14. All of the MS-R antibodies tested were found positive in immunodecoration of amyloid plaques in vivo, although some variability was noted.

# Example 15: Investigation of binding of different monoclonal antibodies to amyloid precursor protein (APP) on the surface of HEK293 cells:

APP is widely expressed in the central nervous system. Binding of antibody to cell surface APP may lead to complement activation and cell destruction in healthy brain areas. Therefore, it is mandatory for therapeutic A-beta antibodies to be devoid of reactivity towards APP. High affinity antibodies against the N-terminal domain of A-beta (e.g. BAP-1, BAP-2) recognize the respective epitope also in the framework of APP. In contrast, the antibodies against the middle epitope (e.g. 4G8), and the antibodies of the invention are surprisingly unable to recognize to cell surface APP. Thus, antibodies of the invention which decorate A-beta, but not APP in vivo, are superior to non-selective antibodies.

The method of flow cytometry is well known in the art. Relative units of fluorescence (FL1-H) measured by flow cytometry indicate cell surface binding of the respective antibody. A fluorescence shift on APP transfected HEK293 compared to untransfected HEK293 cells indicates the unwanted reaction with cell surface APP. As an example, antibodies BAP-1 and BAP-2 against the N-terminal domain show a significant shift of FL-1 signal in HEK293/APP (thick line) compared to untransfected HEK293 cells (dotted line). The 4G8 antibody (specific for the middle A-beta epitope) and all antibodies of the invention (specific for N-terminal and middle A-beta epitopes) show no significant shift in fluorescence. Differences in basal fluorescence between HE293/APP ad HEK293 cells are due to different cell size. A FACScan instrument was used in combination with the Cellquest Pro Software package (both Becton Dickinson).

# Example 16: List of identified SEQ ID NOs relating to inventive antibody molecules

The appended table 10 relates to sequences as defined herein for some specific inventive antibody molecules.

Table 10: Identification of SEQ ID NOs for parental antibodies as well as optimized, matured and/or cross-cloned antibody

molecules

Molecule #	VH prot	VL prot	VH DNA	VL DNA	HCDR3	HCDR3	LCDR3	LCDR3
- 17/1					prot	DNA	prot	DNA
က	4	10	3	6	22		16	15
7	6	12	5	11	24		18	17
8	8	14		13	26		20	19
3.6H5 x 3.6L2	33	47	32		61		75	74
3.6H8 x 3.6L2	35	49	34	48	63		77	76
7.4H2 x 7.2L1	37	51	36		65		79	78
7.9H2 x 7.12L2	39	53	38	52	29	99	81	80
7.9H4 x 7.12L2	41	55	40		69		83	82
7.11H1x7.11L1	43	57	42		71		85	84
7.11H1x7.2L1	45	59	44		73	٠	87	98
7.9H7	89	91	88		93		95	94
3.3H1x3.4L9	295	325	294		355		385	384
3.4H1x3.4L9	297	327	296		357		387	386
3.4H3x3.4L7	299	329	298	328	359		389	388
3.4H3x3.4L9	301	331	300		361		391	390
3.4H7x3.4L9	303	333	302		363		393	392
3.4H7x3.4L7	305	335	304		365		395	394
3.6H5x3.6L1	307	337	306		367		397	396
7.2H2x7.2L1	309	339	308		369		399	398
7.4H2x7.12L2	311	341	310	340	371		401	400
7.9H2x7.2L1	313	343	312		373		403	402
7.9H2x7.12L1	315	345	314	344	375	374	405	404
7.11H2x7.2L1	317	347	316		377	376	407	406
7.11H2x7.9L1	319	349	318		379	378	409	408
7.11H2x7.12L1	321	351	320		381	380	411	410
8.1H1x8.2L1	323	353	322	352	383	382	413	412

#### Claims

- 1. An antibody molecule capable of specifically recognizing two regions of the β-A4 peptide/Aβ4, wherein the first region comprises the amino acid sequence AEFRHDSGY as shown in SEQ ID NO: 1 or a fragment thereof and wherein the second region comprises the amino acid sequence VHHQKLVFFAEDVG as shown in SEQ ID NO: 2 or a fragment thereof.
- 2. The antibody molecule of claim 1, wherein said antibody molecule recognizes at least two consecutive amino acids within the two regions of  $\beta$ -A4.
- 3. The antibody molecule of claim 1 or 2, wherein said antibody molecule recognizes in the first region an amino acid sequence comprising: AEFRHD, EF, EFR, FR, EFRHDSG, EFRHD or HDSG and in the second region an amino acid sequence comprising: HHQKL, LV, LVFFAE, VFFAED or VFFA, FFAEDV.
- 4. The antibody molecule of any one of claims 1 to 3, wherein said antibody molecule comprises a variable V<sub>H</sub>-region as encoded by a nucleic acid molecule as shown in a SEQ ID NO selected from the group consisting of SEQ ID NO: 3, 5 or 7 or a variable V<sub>H</sub>-region as shown in a SEQ ID NO: selected from the group consisting of SEQ ID NOs: 4, 6 and 8.
- 5. The antibody molecule of any one of claims 1 to 3, wherein said antibody molecule comprises a variable V<sub>L</sub>-region as encoded by a nucleic acid molecule as shown in a SEQ ID NO selected from the group consisting of SEQ ID NO: 9, 11 and 13 or a variable V<sub>L</sub>-region as shown in a SEQ ID NO selected from the group consisting of SEQ ID NOs: 10, 12 and 14.

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- 6. The antibody molecule of any one of claims 1 to 5, wherein said antibody molecule comprises at least one CDR3 of an V<sub>L</sub>-region as encoded by a nucleic acid molecule as shown in SEQ ID NOs: 15, 17 or 19 or at least one CDR3 amino acid sequence of an V<sub>L</sub>-region as shown in SEQ ID NOs: 16, 18 or 20 and/or wherein said antibody molecule comprises at least one CDR3 of an V<sub>H</sub>-region as encoded by a nucleic acid molecule as shown in SEQ ID NOs: 21, 23 or 25 or at least one CDR3 amino acid sequence of an V<sub>H</sub>-region as shown in SEQ ID NOs: 22, 24 or 26.
- 7. The antibody molecule of any one of claims 1 to 6, wherein said antibody is selected from the group consisting of MSR-3, -7 and -8 or an affinity-matured version of MSR-3, -7 or -8.
- 8. The antibody molecule of any one of claims 1 to 7, wherein said antibody molecule is a full antibody (immunoglobulin), a F(ab)-fragment, a F(ab)<sub>2</sub>-fragment, a single-chain antibody, a chimeric antibody, a CDR-grafted antibody, a bivalent antibody-construct, a synthetic antibody or a cross-cloned antibody.
- 9. The antibody molecule of any one of claims 1 to 8, wherein said at least two regions of  $\beta$ -A4 form a conformational epitope or a discontinuous epitope.
- 10. The antibody molecule of claim 8 or 9, wherein said cross-cloned antibody is selected from the group consisting of

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MS-R #3.3H1x3.4L9;
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MS-R  $\#3.6H5 \times 3.6L2$ ;

MS-R #3.6H8 x 3.6L2;

MS-R  $\#7.4H2 \times 7.2L1$ ;

MS-R #7.9H2 x 7.12L2;

MS-R #7.9H4 x 7.12L2;

MS-R #7.11H1 x 7.11L1;

MS-R #7.11H1 x 7.2L1:

MS-R  $#3.4H1 \times 3.4L9$ ;

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MS-R #3.4H3 x 3.4L7;
MS-R #3.4H3 x 3.4L9;
MS-R #3.4H7 x 3.4L9;
MS-R #3.4H7 x 3.4L7;
MS-R #3.6H5 x 3.6L1;
MS-R #7.2H2 x 7.2L1;
MS-R #7.4H2 x 7.12L2;
MS-R #7.9H2 x 7.2L1;
MS-R #7.11H2 x 7.12L1;
MS-R #7.11H2 x 7.2L1;
MS-R #7.11H2 x 7.9L1;
MS-R #7.11H2 x 7.12L1 or
MS-R #8.1H1 x 8.2L1.
```

- 11. A nucleic acid molecule encoding an antibody molecule of any one of claims 1 to 10.
- 12. A vector comprising the nucleic acid molecule of claim 11.
- 13. A host cell comprising the vector of claim 12.
- 14. A method for the preparation of an antibody molecule of any one of claims 1 to 10 comprising culturing the host cell of claim 13 under conditions that allow synthesis of said antibody molecule and recovering said antibody molecule from said culture.
- 15. A composition comprising an antibody molecule of any one of claims 1 to 10 or an antibody molecule produced by the method of claim 14.
- 16. The composition of claim 15, which is a pharmaceutical or a diagnostic composition.

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- Use of an antibody molecule of any one of claims 1 to 10 or an antibody molecule produced by the method of claim 14, of a nucleic acid molecule of claim 11, of a vector of claim 12 or a host of claim 13 for the preparation of a
- pharmaceutical composition for the prevention and/or treatment of a disease associated with amyloidogenesis and/or amyloid-plaque formation.
- 18. Use of an antibody molecule of any one of claims 1 to 10 or an antibody
- molecule produced by the method of claim 14 for the preparation of a diagnostic composition for the detection of a disease associated with
  - amyloidogenesis and/or amyloid-plaque formation.
- 19. Use of an antibody molecule of any one of claims 1 to 10 or an antibody molecule produced by the method of claim 14 for the preparation of a pharmaceutical composition for the disintegration of β-amyloid plaques.
- 20. Use of an antibody molecule of any one of claims 1 to 10 or an antibody molecule produced by the method of claim 14 for the preparation of a pharmaceutical composition for passive immunization against  $\beta$ -amyloid plague formation.
- 21. The use of claims 17 or 18, wherein said disease is dementia, Alzheimer's disease, motor neuropathy, Down's syndrome, Creutzfeld Jacob disease, hereditary cerebralhemorrhage with amyloidosis Dutch type, Parkinson's disease, HIV-related dementia, ALS or neuronal disorders related to aging.
- 22. Kit comprising an antibody molecule of any one of claims 1 to 10, a nucleic acid molecule of claim 16, a vector of claim 17 or a host cell of claim 18.
- 23. A method for the optimization of an antibody molecule as defined in any one of claims 1 to 10 comprising the steps of
  - (a) constructing a library of diversified Fab antibody fragments derived from an antibody comprising at least one CDR3 of an V<sub>H</sub>-region as encoded by a nucleic acid molecule as shown in SEQ ID NOs: 21,23 or

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- 25 or at least one CDR3 amino acid sequence of an V<sub>H</sub>-region as shown in SEQ ID NOs: 22, 24 or 26;
- (b) testing the resulting Fab optimization library by panning against  $A\beta/A\beta4$ ;
- (c) identifying optimized clones; and
- (d) expressing of selected, optimized clones.
- 24. The method of claim 23 further comprising a step (ca), whereby the optimized clones are further optimized by cassette mutagenesis
- 25. The method of claim 23 or 24, wherein said A $\beta$ /A $\beta$ 4 in step (b) is aggregated A $\beta$ /A $\beta$ 4.
- 26. The method of any one of claims 23 to 25, wherein said identification in step (c) is carried out by koff-ranking.
- 27. A method for the preparation of a pharmaceutical composition comprising the steps of
  - (a) optimization of an antibody according to the method of any one of claims 23 to 26; and
  - (b) formulating the optimized antibody/antibody molecule with an physiologically acceptable carrier.
- 28. A pharmaceutical composition prepared by the method of claim 27.

Sequence Summary of HuCAL-Fab1 Library

			1/43	Fi	g.	1a		\				
CDR 2	5 0 1 2 3 4 5 6 7 8 9 SanDI	A A S S L C O A S S A C C C C C C C C C C C C C C C C	A A A A A A A A A A A A A A A A A A A	198	CDR 2	6 b c 3 4 5 6 7 8 9 0	- I F G T A N Y A	A N S G G T N Y A	- WDDDKYYS	N A N L S D S A	- GDSDTRYS	R - S K W Y N D Y A
Framework 2	5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 Kpnl SexAl Asel	0 0 0 0 1 0 7 7 7	W Y Q Q R H P G G P P K L L I Y Y W Y Q Q R H P G G R H P K L M I Y Y Q Q R H P G G R H P K L M I Y Y Q Q R H P G G R H P K L M I X Y Q Q R H P G G R H P K L M I X Y Q Q R H P G G R H P K L M I X Y Q Q R H P G G R H P K L M I X Y Q Q R H P G G R H P R K L M I X Y Q Q R H P G G R H P R K L M I X Y Q Q Q Q R H P R K L M I X Y Q Q Q Q R H P R K L M I X Y Q Q Q Q R H P R K L M I X Y Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q	KpnI XmaI Bbet	Framework 2	2 3 4	Anol Anol Anol Anol Q G L E W M G G I I P	A P G C L E	Q Y P G K A L E W L A L I D Q A P G K G L E W V S A I S G.	QPPGKGLEWIGYIY-	QMPGKGLEWMGIIY.P	QSPGRGLEWLGRTYY
CDR 1	3 67890abcdef1234	G I S S Y L A S L L H S - N G Y N Y L D S V S S S Y L A	S Q S V L Y S S N N K N Y L A S S N 1 G S V N Y V S T S S D V G G Y K Y A S D A L G D K Y A S S		CDR1	3 5678901ab2345678 <sub>poet</sub>	G G T F S S Y A I S W V R		GFT FSS YAMS WVR	GGSISS YYWSWIR	GYSFTS YWIGWVR	GDSVSSNSAAWNWIR
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Fig. 1b

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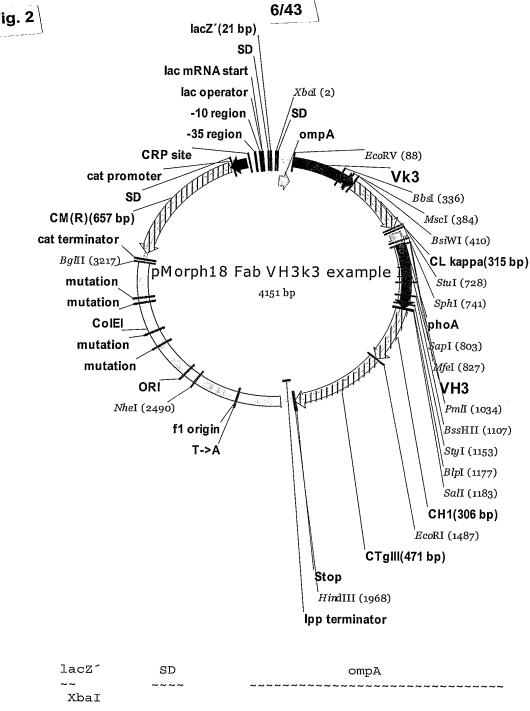
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GGG AAA GCC CTC GAG TGG CTG GCT CTG ATT GAT	TGG GAT GAT GAT ANG TAT TAT AGC ACC AGC CTG AAA ACG CGT CTG ACC ATT AGC AAA GAT AGT TCG AAA AAT CAG GTG CTG ACT ATG ACC	AAC
AAG GGT CTC GAG TGG GTG AGC GCG	AAC	AGC
GGG AAG GGT CTC GAG TGG ATT GGC TAT ATT TAT	TAT AGC GGC AGC ACC AAC TAT AAT CCG AGC CTG AAA AGC CGG GTG ACC ATT AGC GTT GAT AGT TCG AAA AAC CAG TTT AGC CTG AAA CTG AGC	AGC
AAG GGT CTC GAG TGG ATG GGC ATT	GGC GAT AGC GAT ACC CGT TAT TCT CCG AGC TTT CAG GGC CAG GTG ACC ATT AGC GCG GAT AAA AGC ATT AGC ACC GCG TAT CTT CAA TGG AGC	AGC
GGG CGT GGC CTC GAG TGG CTG GGC CGT ACC 1	ACC TAT TAT CGT - AGC AAA TGG TAT AAC GAT TAT GCG GTG AGC GTG AAA AGC CGG ATT ACC ATC AAC CCG GAT AQT TCG AAA AAC CAG TTT AGC CTG CAA CTG AAC AA	AGC

2 3 BipI AGC TCA GC AGC TCA GC AGC TCA GC AGC TCA GC AGC TCA GC AGC TCA GC

Fig. 1b cont.

## 5 6 7 8 9 9 1 2 3 4 5 9 9 1 2 3 4 5 9 9 1 2 3 4 5 9 9 1 2 3 4 5 6 7 8 9 9 1 2 3 4 5 6 7 8 9 9 1 2 3 4 5 6 7 8 9 9 1 2 3 4 5 6 7 8 9 9 1 2 3 4 5 6 7 8 9 9 1 2 3 4 5 6 7 8 9 9 1 2 3 4 5 6 7 8 9 9 1 2 3 4 5 6 7 8 9 9 1 2 3 4 5 6 7 8 9 9 1 2 3 4 5 6 7 8 9 9 1 2 3 4 5 6 7 8 9 9 1 2 3 4 5 6 7 8 9 9 1 2 3 4 5 6 7 8 9 9 1 2 3 4 5 6 7 8 9 9 1 2 3 4 5 6 7 8 9 9 1 2 3 4 5 6 7 8 9 9 1 2 3 4 5 6 7 8 9 9 1 2 3 4 5 6 7 8 9 9 1 2 3 4 5 6 7 8 9 9 0 1 2 3 4 5 6 7 8 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9												Framework 4	11	3 4 5 6 7 8 9 0 1 2	IAS	TGG GGC CAA GGC ACC CTG GTG ACG GTT AG	тве вфс сла вфс лсс ств вте лсв втт дв	тве вес сла вес дсс ств втв дсв втт дв	тве вес сла вес дсс сте вте дсе втт дв	тве вес сла вес лсс ств вте дсе втт дв	TGG GGC CAA GGC ACC CTG GTG ACG GTT AG	тев вес сла вес до ств втв де втт дв
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S   6   7   8   9   0				×	×	×	×							6	r	<u>9</u>	1916	įξ.	910	916	ATG	<u>9</u> 6
5 6 7 8  ACT/GTT TAT TAT TGC  GTG TAT TAT TGC  GTG TAT TAT TGC  GAT TAT TAT TGC  GAT TAT TAT TGC  GAT TAT TAT TGC  GAT TGC  GAT TAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT TAT TGC  GAT TAT  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC  GAT TAT TGC		6	0	8	Se	8	CAG	덜	5	널				œ	EagI		ပ္ပ	မ္ဗ	ည္ပ	ပ္ပပ္ပ	ည	ပ္ပ
5 6  ACT/GTT TAT GTG TAT GTG TAT GAT TAT GAT TAT GAT TAT GAT TAT GAT AGC CGT AGC CGT AGC CGT AGC ACG AAC AAC AAC AAC AAC AAC AAC AAC			6	L	×			8 8	8	8				7	Ĺ		ន្ត	٩Į	<del>S</del>	ă	å	ଧି
5 6  ACT/GTT TAT GTG TAT GTG TAT GAT TAT GAT TAT GAT TAT GAT TAT GAT AGC CGT AGC CGT AGC CGT AGC ACG AAC AAC AAC AAC AAC AAC AAC AAC			8	55	75	707	760	75	5	75				9		GAT	A GAT	GAT	\ GAT	GAT	GAT	GAT
5 ACT/GIT GIG GAT GAT GAT GAT GAT GAT GAT GAT AAA AACG AAA AAA AACC			7	T TA	T TA	TAT		T TA	T TA					ហ		C GA	C GA	6 67	G 6A		G AG	Ø.
6CG ACT/GI GGC GTG GGC GTG GGG GAT GGG GAT GGG GAT GGG GGT CTG GGT CTG CGT CTG CGT			9											4		AĞ						8
600 600 600 600 600 600 600 600 600 600			5	ACT/GT	GTG	ACT/GI	GTG	GAT	GAT	GAT				3		CGT	ल	GAC	लु	ACG	AW	ACC
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GGCACTGGCT GGTTTCGCTA CCGTAGCGCA GGCCGATATC GTGCTGACCC

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7/43 | Fig. 2 cont. CCGTGACCGA CCAAAGCGAT GGCATCGCGT CCGGCTATAG CACGACTGGG Vk3 · S P A T L S L S P G E R A T L S AGAGCCCGGC GACCCTGAGC CTGTCTCCGG GCGAACGTGC GACCCTGAGC 101 TCTCGGGCCG CTGGGACTCG GACAGAGGCC CGCTTGCACG CTGGGACTCG CRASQSVSSSYLAWYQQ 151 TGCAGAGCGA GCCAGAGCGT GAGCAGCAGC TATCTGGCGT GGTACCAGCA ACGTCTCGCT CGGTCTCGCA CTCGTCGTCG ATAGACCGCA CCATGGTCGT 77k-3 · K P G Q A P R L L I Y G A S S R A · GAAACCAGGT CAAGCACCGC GTCTATTAAT TTATGGCGCG AGCAGCCGTG 201 CTTTGGTCCA GTTCGTGGCG CAGATAATTA AATACCGCGC TCGTCGGCAC Vk3 · T G V P A R F S G S G S G T D F 251 CAACTGGGGT CCCGGCGCGT TTTAGCGGCT CTGGATCCGG CACGGATTTT GTTGACCCCA GGGCCGCGCA AAATCGCCGA GACCTAGGCC GTGCCTAAAA Vk3 BbsI TLTISSLEPEDFAV YYC. 301 ACCTGACCA TTAGCAGCCT GGAACCTGAA GACTTTGCGG TGTATTATTG TGGGACTGGT AATCGTCGGA CCTTGGACTT CTGAAACGCC ACATAATAAC Vk3 · Q Q H Y T T P P T F G Q G T K V E · CCAGCAGCAT TATACCACCC CGCCGACCTT TGGCCAGGGT ACGAAAGTTG 351 GGTCGTCGTA ATATGGTGGG GCGGCTGGAA ACCGGTCCCA TGCTTTCAAC CL kappa Vk3 BsiWI · I K R T V A A P S V F I F P P S AAATTAAACG TACGGTGGCT GCTCCGAGCG TGTTTATTTT TCCGCCGAGC 401 TTTAATTTGC ATGCCACCGA CGAGGCTCGC ACAAATAAAA AGGCGGCTCG CL kappa DEQL KSG TAS V V C L L N N 451 GATGAACAAC TGAAAAGCGG CACGGCGAGC GTGGTGTGCC TGCTGAACAA CTACTTGTTG ACTTTTCGCC GTGCCGCTCG CACCACGCG ACGACTTGTT CL kappa · F Y P R E A K V Q W K V D N A L Q · 501 CTTTTATCCG CGTGAAGCGA AAGTTCAGTG GAAAGTAGAC AACGCGCTGC GAAAATAGGC GCACTTCGCT TTCAAGTCAC CTTTCATCTG TTGCGCGACG СЬ карра  $\cdot$  s g n s q e s v t e q d s k d s AAAGCGGCAA CAGCCAGGAA AGCGTGACCG AACAGGATAG CAAAGATAGC 551 TTTCGCCGTT GTCGGTCCTT TCGCACTGGC TTGTCCTATC GTTTCTATCG CL kappa 

		8/43	Fig. 2 co	nt. \
601	T Y S L S S T ACCTATTCTC TGAGCAGCAC TGGATAAGAG ACTCGTCGTG	CCTGACCCTG		ATTATGAAAA
651	· H K V Y A C I ACATAAAGTG TATGCGTGCG TGTATTTCAC ATACGCACGC CL kappa	AAGTGACCCA TTCACTGGGT	TCAAGGTCTG .	AGCAGCCCGG
701	· T K S F N R TGACTAAATC TTTTAATCGT ACTGATTTAG AAAATTAGCA	StuI ~~~~~ G E A GGCGAGGCCT	GATAAGCATG (	
751	M K Q S T AATAAAATGA AACAAAGCAC TTATTTTACT TTGTTTCGTG	I A L	GCACTCTTAC (	CGTTGCTCTT
	phoA SapI	MfeI	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	~~~~~~
801	CACCCCTGTT ACCAAAGCCG GTGGGGACAA TGGTTTCGGC	V Q L AAGTGCAATT TTCACGTTAA VH3	V E S GGTGGAAAGC (	GGGGGGGGG GGGGGGGGGG
851	· V Q P G G S TGGTGCAACC GGGCGGCAGC ACCACGTTGG CCCGCCGTCG	L R L S	C A A GCTGCGCGGC (	S G F CTCCGGATTT
901	T F S S Y A M ACCTTTAGCA GCTATGCGAT TGGAAATCGT CGATACGCTA	GAGCTGGGTG	R Q A P CGCCAAGCCC C GCGGTTCGGG C	CTGGGAAGGG
951	· L E W V S A I TCTCGAGTGG GTGAGCGCGA AGAGCTCACC CACTCGCGCT	TTAGCGGTAG	CGGCGGCAGC A	
	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	.~~~~~~~~~~	PmlI	. ~ ~ ~ ~ ~ ~ ~ ~ ~
1001	· D S V K G R CGGATAGCGT GAAAGGCCGT GCCTATCGCA CTTTCCGGCA	TTTACCATTT AAATGGTAAA VH3		TCGAAAAAC
1051	T L Y L Q M N ACCCTGTATC TGCAAATGAA TGGGACATAG ACGTTTACTT	S L R CAGCCTGCGT	GCGGAAGATA C	GGCCGTGTA
	BssHII	~~~~~~~~	~~~~~~~~~~~	

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	9/43 Fig. 2 cont.		
1101	· Y C A R W G G D G F Y A M D T TTATTGCGCG CGTTGGGGCG GCGATGGCTT TTATGCGATG GATT AATAACGCGC GCAACCCCGC CGCTACCGAA AATACGCTAC CTAA CH1	ATTGGG	•
	VH3	~~~~	
	SalI		
	StyI BlpI		
1151	· Q G T L V T V S S A S T K G GCCAAGGCAC CCTGGTGACG GTTAGCTCAG CGTCGACCAA AGGTCCGGTTCCGTG GGACCACTGC CAATCGAGTC GCAGCTGGTT TCCACCH1	CCAAGC	
1201	V F P L A P S S K S T S G G T GTGTTTCCGC TGGCTCCGAG CAGCAAAAGC ACCAGCGGCG CCCCCCCCCC	GGCTGC	
1251	· L G C L V K D Y F P E P V T V CCTGGGCTGC CTGGTTAAAG ATTATTTCCC GGAACCAGTC ACCG GGACCCGACG GACCAATTTC TAATAAAGGG CCTTGGTCAG TGGCA	IGAGCT	•
1301	· N S G A L T S G V H T F P A  GGAACAGCGG GGCGCTGACC AGCGGCGTGC ATACCTTTCC GGCGC  CCTTGTCGCC CCGCGACTGG TCGCCGCACG TATGGAAAGG CCGCC  CH1	GTGCTG	
1351	Q S S G L Y S L S S V V T V P CAAAGCAGCG GCCTGTATAG CCTGAGCAGC GTTGTGACCG TGCCC GTTTCGTCGC CGGACATATC GGACTCGTCG CAACACTGGC ACGGC CH1	GAGCAG	
1401		CGAGCA	•
	EcoRI	~~~~	
1451	T K V D K K V E P K S E F G ACACCAAAGT GGATAAAAAA GTGGAACCGA AAAGCGAATT CGGGG TGTGGTTTCA CCTATTTTTT CACCTTGGCT TTTCGCTTAA GCCCCCTGIII	GGAGGG CCTCCC	
1501	S G S G D F D Y E K M A N A N A N A AGCGGGAGCG GTGATTTGA TTATGAAAAG ATGGCAAACG CTAATTCGCCCTCGC CACTAAAACT AATACTTTTC TACCGTTTGC GATTACTGIII	K G · FAAGGG	
1551	· A M T E N A D E N A L Q S D A GGCTATGACC GAAAATGCCG ATGAAAACGC GCTACAGTCT GACGC CCGATACTGG CTTTTACGGC TACTTTTGCG CGATGTCAGA CTGCC CTGIII	CTAAAG GATTTC	•
1601	· K L D S V A T D Y G A A I D GCAAACTTGA TTCTGTCGCT ACTGATTACG GTGCTGCTAT CGATG	G F	

			10/43	Fig. 2 con	it.
	CGTTTGAACT	AAGACAGCGA	TGACTAATGC CTgIII	CACGACGATA	GCTACCAAAG
1651		V S G L TTTCCGGCCT AAAGGCCGGA	TGCTAATGGT	AATGGTGCTA	
1701	TGCTGGCTCT	N S Q I AATTCCCAAA TTAAGGGTTT	TGGCTCAAGT	$\tt CGGTGACGGT$	
1751		N F R TAATTTCCGT ATTAAAGGCA		CTTCCCTCCC	TCAATCGGTT
1801	GAATGTCGCC	F V F CTTTTGTCTT GAAAACAGAA	TGGCGCTGGT	AAACCATATG	
1851	TGATTGTGAC	K I N I AAAATAAACT TTTTATTTGA	TATTCCGTGG	TGTCTTTGCG	
1901	ATGTTGCCAC	F M Y CTTTATGTAT GAAATACATA	GTATTTTCTA	CGTTTGCTAA	CATACTGCGT
	~~~~~~~~~~	Stop	_	p terminato	
		Hind		. ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~	-~~~~
1951		CTTGATAAGC GAACTATTCG			
2001		TTTTTTTGTC AAAAAAACAG			TTGCAATTAT
2051		ATTCGCGTTA TAAGCGCAAT		AAATCAGCTC	ATTTTTTAAC
2101		f1 AAATCGGCAA TTTAGCCGTT			
2151	CTATCCCAAC	f1 AGTGTTGTTC TCACAACAAG			
2201	ACGTGGACTC	f1 CAACGTCAAA	l origin GGGCGAAAAA	CCGTCTATCA	GGGCGATGGC

11/43 fl origin

Fig. 2 cont.

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2251			CTAATCAAGT GATTAGTTCA			
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2301	יייא א א פיפא פיייא		1 origin CTAAAGGGAG		a ca commoa c	
23 U.L			GATTTCCCTC			
	~~~~~~~	~~~~~~~~	~~~~~~~		~~~~~~~	
			1 origin			
2351			GCGAGAAAGG			
	CCCCTTTCGG	CCGCTTGCAC	CGCTCTTTCC	TTCCCTTCTT	TCGCTTTCCT	
	~~~~~~~		~~~~~~~~ 1 origin	~~~~~~~	~~~~~~~	
2401	GCGGGCGCTA		AAGTGTAGCG	GTCACGCTGC	GCGTAACCAC	
			TTCACATCGC			
	~~~~~~~	~~~~~~~~	~~~~~~~~	~~~~~~	~~~~~~~~	
		£	1 origin			
				N]	heI	
2451	מא מא ממממממ	ርርርርምም <i>እ እ</i> ምር	CGCCGCTACA	~~.	~~~~ ~~~~	
2431		- · · ·	GCGGCGATGT			
	~~~~~~~	~~~~~~~	~~~~~~~~~	~~~~~~	~~	
		f1 orig			ColEI	
2501			CCAGGAACCG			
	TCGTTTTCCG		GGTCCTTGGC	ATTTTTCCGG	CGCAACGACC	
	~~~~~~~	~~~~~~~~~	~~~~~~~ ColEI	~~~~~~~~	~~~~~~~	
	ORI		COIEI			
2551	CGTTTTTCCA	TAGGCTCCGC	CCCCTGACG	AGCATCACAA	AAATCGACGC	
			GGGGGACTGC			
	~~~~~~~		~~~~~~~~~		~~~~~~~	
			ColEI			
2601			CCCGACAGGA GGGCTGTCCT			
	AGIICAGICI		~~~~~~~~~~		IGGICCGCAA	
			ColEI			
2651	TCCCCCTGGA	AGCTCCCTCG	TGCGCTCTCC	TGTTCCGACC	CTGCCGCTTA	
	AGGGGGACCT	TCGAGGGAGC	ACGCGAGAGG	ACAAGGCTGG	GACGGCGAAT	
	~~~~~~~		Colei	~~~~~~~~	~~~~~~~	
			COLET		mı	ıtation
					~	
2701	CCGGATACCT	GTCCGCCTTT	CTCCCTTCGG	GAAGCGTGGC	GCTTTCTCAT	
	GGCCTATGGA	CAGGCGGAAA	GAGGGAAGCC	CTTCGCACCG	CGAAAGAGTA	
	~~~~~~~			·~~~~~~~~	~~~~~~	
	mutation		ColEI			
	~					
2751	AGCTCACGCT	GTAGGTATCT	CAGTTCGGTG	TAGGTCGTTC	GCTCCAAGCT	
	TCGAGTGCGA	CATCCATAGA	GTCAAGCCAC	ATCCAGCAAG	CGAGGTTCGA	
	~~~~~~~	~~~~~~~	~~~~~~~~		~~~~~~~	
			Colei	- n + d		
			mut	cation		
2801	GGGCTGTGTG	CACGAACCCC	~ CCGTTCAGTC	CGACCGCTGC	GCCTTATCCG	
			GGCAAGTCAG			
	~~~~~~~~			~~~~~~~	~~~~~~~	
			ColEI			

			12/43	Fig. 2 c	ont.
2851				GACACGACTT CTGTGCTGAA	
2901				GCGAGGTATG CGCTCCATAC	
	~~~~~~~~		ColEI		mutation
2951				CGGCTACACT GCCGATGTGA	
			ColEI mutation	n	
3001				TTACCTTCGG AATGGAAGCC	
3051				GCTGGTAGCG CGACCATCGC	
3101				AAAAGGATCT TTTTCCTAGA	
3151				AGTGGAACGA TCACCTTGCT	
	~~~~~~~	~~~~~~~~	ColEI	~~~~~	
				cat te	rminator
		BglII ~~~~~	~~	~~~~~~	~~~~~~
3201				CGTTTAAGGG GCAAATTCCC	
	ColEI cat termina	ator			
3251		TTTAATGCGG		ACTCATCGCA TGAGTAGCGT	
				M(R)	
3301				CCATCACAAA GGTAGTGTTT	
3351				GTCGCCTTGC CAGCGGAACG	
3401				TGTCCATATT ACAGGTATAA	
3451				TTGGCTGAGA AACCGACTCT	

			13/43	Fig. 2 con	4
			CM(R)	1 ig. 2 con	L.
3501			GGAAATAGGC	CAGGTTTTCA GTCCAAAAGT	
	~~~~~~~~~~		CM (R)	~~~~~~~	~~~~~~
3551			TGTAGAAACT	GCCGGAAATC CGGCCTTTAG	
			CM (R)	~~~~~~~~~	~~~~~~~
3601			CGTTTCAGTT	TGCTCATGGA ACGAGTACCT	
			CM (R)	~~~~~~~	~~~~~~~
3651			ATATCACCAG	CTCACCGTCT GAGTGGCAGA	
	~~~~~~~~	~~~~~~~~~	CM(R)	~~~~~~	~~~~~~
3701			TTCATCAGGC	GGGCAAGAAT CCCGTTCTTA	
			CM(R)	~~~~~~~~~	~~~~~~
3751			ATTTTTCTTT	ACGGTCTTTA TGCCAGAAAT	
	~~~~~~~~		CM(R)	~~~~~~~~~	~~~~~~
3801			GGTTATAGGT	ACATTGAGCA TGTAACTCGT	
	~~~~~~~	. ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ .	CM (R)	~~~~~~~~~	~~~~~~
3851			CGATGCCATT	GGGATATATC CCCTATATAG	
			CM(R)		~~~~~
3901	ATAGGTCACT		CATTTTAGCT	TCCTTAGCTC AGGAATCGAG	
		1 (R)	~~~	SD	
			~~~~~~		
				cat promotes	
3951	CGATAACTCA GCTATTGAGT			TCTTATTTCA AGAATAAAGT	
		cat	promoter		
				RP site	
4001		AGTGGGCTGC	TCTAATGTGA	GTTAGCTCAC CAATCGAGTG	TCATTAGGCA
	cat promote				
	start				lac mRNA
					lac operator
	-35	region		-10 regio	on
4051	იიი <u>გი</u> შელო ~~~	~~~~ TACACMMMAM	COTTO COCO	~~~~~~ CCTA TO CTTO CTT	ርጥርርን አጥጥርመ
±05T		ATGTGAAATA or		CGTATGTTGT GCATACAACA lac	CACCTTAACA
4101				CTATGACCAT	GATTACGAAT

14/43

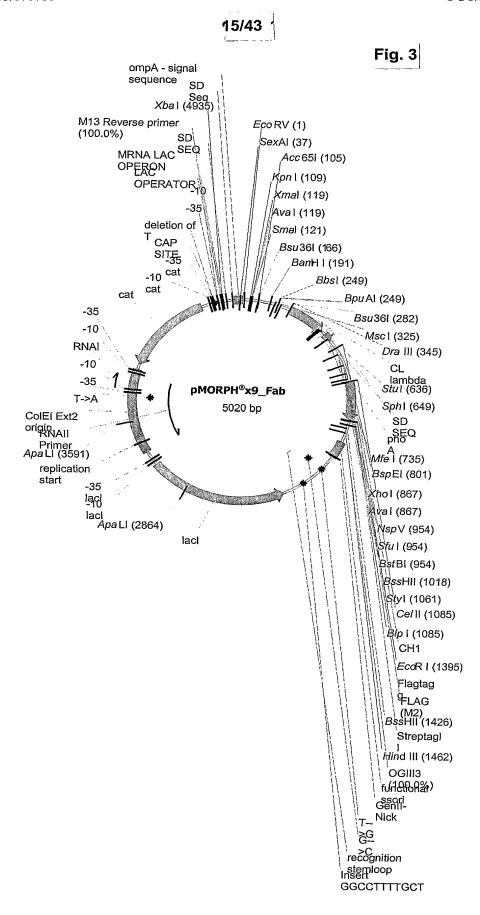
Fig. 2 cont.

CTCGCCTATT GTTAAAGTGT GTCCTTTGTC GATACTGGTA CTAATGCTTA

lacZ´

4151 T

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PCT/EP03/01759 Fig. 3 cont. 16/43 SexAI EcoRV ~~~ ATCGTGCTGA CCCAGCCGCC TTCAGTGAGT GGCGCACCAG GTCAGCGTGT TAGCACGACT GGGTCGGCGG AAGTCACTCA CCGCGTGGTC CAGTCGCACA GACCATCTCG TGTAGCGGCA GCAGCAGCAA CATTGGCAGC AACTATGTGA CTGGTAGAGC ACATCGCCGT CGTCGTCGTT GTAACCGTCG TTGATACACT XmaI ~~~~~ SmaI KpnI ~~~~~ ~~~~~ Acc65I AvaI ~~~~~ 101 GCTGGTACCA GCAGTTGCCC GGGACGGCGC CGAAACTGCT GATTTATGAT CGACCATGGT CGTCAACGGG CCCTGCCGCG GCTTTGACGA CTAAATACTA Bsu36I BamHI ~~~~~ AACAACCAGC GTCCCTCAGG CGTGCCGGAT CGTTTTAGCG GATCCAAAAG 151 TTGTTGGTCG CAGGGAGTCC GCACGGCCTA GCAAAATCGC CTAGGTTTTC BpuAI ~~~~~ BbsI CGGCACCAGC GCGAGCCTTG CGATTACGGG CCTGCAAAGC GAAGACGAAG 201 GCCGTGGTCG CGCTCGGAAC GCTAATGCCC GGACGTTTCG CTTCTGCTTC Bsu36I CGGATTATTA TTGCCAGAGC TATGACATGC CTCAGGCTGT GTTTGGCGGC 251 GCCTAATAAT AACGGTCTCG ATACTGTACG GAGTCCGACA CAAACCGCCG DraIII MscI GGCACGAAGT TTAACCGTTC TTGGCCAGCC GAAAGCCGCA CCGAGTGTGA 301 CCGTGCTTCA AATTGGCAAG AACCGGTCGG CTTTCGGCGT GGCTCACACT CGCTGTTTCC GCCGAGCAGC GAAGAATTGC AGGCGAACAA AGCGACCCTG GCGACAAAGG CGGCTCGTCG CTTCTTAACG TCCGCTTGTT TCGCTGGGAC GTGTGCCTGA TTAGCGACTT TTATCCGGGA GCCGTGACAG TGGCCTGGAA 401 CACACGGACT AATCGCTGAA AATAGGCCCT CGGCACTGTC ACCGGACCTT GGCAGATAGC AGCCCCGTCA AGGCGGGAGT GGAGACCACC ACACCCTCCA 451 CCGTCTATCG TCGGGGCAGT TCCGCCCTCA CCTCTGGTGG TGTGGGAGGT AACAAAGCAA CAACAAGTAC GCGGCCAGCA GCTATCTGAG CCTGACGCCT 501 TTGTTTCGTT GTTGTTCATG CGCCGGTCGT CGATAGACTC GGACTGCGGA

GAGCAGTGGA AGTCCCACAG AAGCTACAGC TGCCAGGTCA CGCATGAGGG

CTCGTCACCT TCAGGGTGTC TTCGATGTCG ACGGTCCAGT GCGTACTCCC

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		17	IA3 Fig	. 3 cont.	
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601			TTGCGCCGAC		
	CTCGTGGCAC	CTTTTTTGGC	AACGCGGCTG	ACTCCGGACT	ATTCGTACGC
651				TTGCACTGGC AACGTGACCG	
	ATCCICITIT	AIIIIACIII	GIIICGIGAI	AACGIGACCG	IGAGAAIGGC
				MfeI	
				~~~~~	
701				GTGCAATTGA	
	AACGAGAAGT	GGGGACAATG	GTTTCGGGTC	CACGTTAACT	TTCTTTCGCC
					BspEI
					~
751	CCCGGCCCTG	GTGAAACCGA	CCCAAACCCT	GACCCTGACC	TGTACCTTTT
	GGGCCGGGAC	CACTTTGGCT	GGGTTTGGGA	CTGGGACTGG	ACATGGAAAA
	D EIT				
	BspEI				
801	CCGGATTTAG	CCTGTCCACG	TCTGGCGTTG	GCGTGGGCTG	GATTCGCCAG
				CGCACCCGAC	
		XhoI			
		~~~~~ AvaI	~~		
		~~~~	<b>~</b> ~		
851	CCGCCTGGGA	AAGCCCTCGA	GTGGCTGGCT	CTGATTGATT	GGGATGATGA
	GGCGGACCCT	TTCGGGAGCT	CACCGACCGA	GACTAACTAA	CCCTACTACT
001		7 667 667 666		ECECA CCA EIII	7 C C 7 7 7 C 7 C 7 C 7
901			TGAAAACGCG	AGACTGGTAA	AGCAAAGATA
	ATICATAATA	1001001000	ACTITIOCOC	AGACIGGIZZI	10011101111
	BstBI				
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	SfuI				
	NapV	•			
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951	CTTCGAAAAA	TCAGGTGGTG	CTGACTATGA	CCAACATGGA	CCCGGTGGAT
	GAAGCTTTTT	AGTCCACCAC	GACTGATACT	GGTTGTACCT	GGGCCACCTA
		D = = II	r -r		
		BssHI ~~~~			
1001	ACGGCCACCT	ATTATTGCGC	GCGTTCTCCT	CGTTATCGTG	GTGCTTTTGA
	TGCCGGTGGA	TAATAACGCG	CGCAAGAGGA	GCAATAGCAC	CACGAAAACT
				BlpI ~~~~~	
		StyI		CelII	
	~~	~~~~		~~~~~	
1051				TAGCTCAGCG	
	AATAACCCCG	GTTCCGTGGG	ACCACTGCCA	ATCGAGTCGC	AGCTGGTTTC

	18/43 Fig. 3 cont.										
1101				GCAAAAGCAC CGTTTTCGTG							
1151				TATTTCCCGG ATAAAGGGCC							
1201				CGGCGTGCAT GCCGCACGTA							
1251			CTGTATAGCC GACATATCGG	TGAGCAGCGT ACTCGTCGCA	TGTGACCGTG ACACTGGCAC						
1301				ATTTGCAACG TAAACGTTGC							
					EcoRI						
1351				GGAACCGAAA CCTTGGCTTT							
			BssHII								
1401			AAAGGCGCGC TTTCCGCGCG	CGTGGAGCCA GCACCTCGGT	CCCGCAGTTT GGGCGTCAAA						
		HindIII									
1451		TTCGAACTGG		AAAATGGCGC TTTTACCGCG							
1501				GGGGGGGGG CCCCCCCC							
1551				ATATTTTGTT TATAAAACAA							
1601				AACCAATAGG TTGGTTATCC							
1651	CAAAATCCCT GTTTTAGGGA			CGAGATAGGG GCTCTATCCC							
1701	TTCCAGTTTG AAGGTCAAAC			AGAACGTGGA TCTTGCACCT							
1751				GGCCCACTAC CCGGGTGATG							
1801	ACCCTAATCA TGGGATTAGT			CCGTAAAGCA GGCATTTCGT							

19/43 Fig. 3 cont.

		101			
1851	ACCCTAAAGG TGGGATTTCC	GAGCCCCCGA CTCGGGGGCT	TTTAGAGCTT AAATCTCGAA	GACGGGGAAA CTGCCCCTTT	GCCGGCGAAC CGGCCGCTTG
1901	GTGGCGAGAA	AGGAAGGGAA	GAAAGCGAAA	GGAGCGGGCG	CTAGGGCGCT
	CACCGCTCTT	TCCTTCCCTT	CTTTCGCTTT	CCTCGCCCGC	GATCCCGCGA
1951		GCGGTCACGC CGCCAGTGCG		CACCACACCC GTGGTGTGGG	
2001		ACAGGGCGCG TGTCCCGCGC	TGCTAGACTA ACGATCTGAT		
2051		GTGGGCTGCA CACCCGACGT	AAACAAAACG TTTGTTTTGC		
2101		TAGCCTCACT ATCGGAGTGA			
2151		CAGTGAATCG GTCACTTAGC			GGTTTGCGTA CCAAACGCAT
2201		GGGTGGTTTT CCCACCAAAA			GCAACAGCTG CGTTGTCGAC
2251		ACCGCCTGGC TGGCGGACCG			CGGTCCACGC GCCAGGTGCG
2301		CAGCAGGCGA GTCGTCCGCT			CAGCGGCGGG GTCGCCGCCC
2351		AGCTGTCCTC TCGACAGGAG			CCGAGATGTC GGCTCTACAG
2401		CGCAGCCCGG GCGTCGGGCC			
2451		GTTGGCAACC CAACCGTTGG			
2501		TGGTTTGTTG ACCAAACAAC			
2551		ATCGGCTGAA TAGCCGACTT			
2601		CAGACGCGCC GTCTGCGCGG			
2651		GGTGGCCCAA CCACCGGGTT			CCAGTCGCGT GGTCAGCGCA
2701					TGGTCAGAGA ACCAGTCTCT

		20	0/43 \	Fig. 3 cont.	
2751				AGGCAGCTTC	
	GTAGTTCTTT	ATTGCGGCCT	TGTAATCACG	TCCGTCGAAG	GTGTCGTTAT
2801	GCATCCTGGT	CATCCAGCGG	ATAGTTAATA	ATCAGCCCAC	TGACACGTTG
	CGTAGGACCA	GTAGGTCGCC	TATCAATTAT	TAGTCGGGTG	ACTGTGCAAC
		ApaLI			
0051	CGCGAGAAGA		CCCCTTTTACA	GGCTTCGACG	ССССТТССТТ
2851		AACACGTGGC		CCGAAGCTGC	
2901	CTACCATCGA	CACGACCACG	CTGGCACCCA	GTTGATCGGC	GCGAGATTTA
	GATGGTAGCT	GTGCTGGTGC	GACCGTGGGT	CAACTAGCCG	CGCTCTAAAT
2951	ATCCCCCCCA	$C\Delta\Delta$ TTTCCCA	CGGCGCGTGC	AGGGCCAGAC	TGGAGGTGGC
2731				TCCCGGTCTG	
					~~~~~~~~~
3001			GTTTGCCCGC	CAGTTGTTGT GTCAACAACA	
	TIGCGGIIAG	ICGIIGCIGA	CAAACGGGGG	GICAMCANCA	CCCICCCII
3051	TAGGAATGTA		GCCATCGCCG		TTCCCGCGTT
	ATCCTTACAT	TAAGTCGAGG	CGGTAGCGGC	GAAGGTGAAA	AAGGGCGCAA
3101	TTCGCAGAAA	CGTGGCTGGC	CTGGTTCACC	ACGCGGGAAA	CGGTCTGATA
	AAGCGTCTTT	GCACCGACCG	GACCAAGTGG	TGCGCCCTTT	GCCAGACTAT
21 - 1	3 C3 C3 C3 CCC	GCATACTCTG	CGACATCGTA	<b>ጥ</b> እ ሮረጥጥን ሮጥ	GGTTTCACAT
3151		CGTATGAGAC	GCTGTAGCAT	ATTGCAATGA	
3201		GAATTGACTC	TCTTCCGGGC		CATACCGCGA GTATGGCGCT
	AGTGGTGGGA	CTTAACTGAG	AGAAGGCCCG	CGATAGIACG	GIAIGGCGCI
3251		GCCATTCGAT			GCCAGCAAAA
	TTCCAAAACG	CGGTAAGCTA	CGATCGGTAC	ACTCGTTTTC	CGGTCGTTTT
3301	GGCCAGGAAC	CGTAAAAAGG	CCGCGTTGCT	GGCGTTTTTC	CATAGGCTCC
3301				CCGCAAAAAG	
0054	GGGGGGG		*************************		
3351				CGAGTTCAGT	GAGGTGGCGA CTCCACCGCT
	0000001101	00100111010	111111111111111111111111111111111111111	001101101101	
3401				TTTCCCCCTG	
	TTGGGCTGTC	CTGATATTTC	TATGGTCCGC	AAAGGGGGAC	C'I"I'CGAGGGA
3451	CGTGCGCTCT	CCTGTTCCGA	CCCTGCCGCT	TACCGGATAC	CTGTCCGCCT
	GCACGCGAGA	GGACAAGGCT	GGGACGGCGA	ATGGCCTATG	GACAGGCGGA
3501	դփնփնննարու	GGGAAGCGTG	GCGCTTTC	ATAGCTCACG	CTGTAGGTAT
2001				TATCGAGTGC	
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3551	CTCAGTTCGG	TGTAGGTCGT	TCGCTCCAAG	CTGGGCTGTG	TGCACGAACC
	GAGTCAAGCC	ACATCCAGCA	AGCGAGGTTC	GACCCGACAC	ACGTGCTTGG

		21	1/43	Fig. 3 cont.	
3601			GCGCCTTATC CGCGGAATAG		
3651		AAGACACGAC TTCTGTGCTG	TTATCGCCAC AATAGCGGTG	TGGCAGCAGC ACCGTCGTCG	
3701		GAGCGAGGTA CTCGCTCCAT	TGTAGGCGGT ACATCCGCCA	0011101101	TCTTGAAGTG AGAACTTCAC
3751	GTGGCCTAAC CACCGGATTG	TACGGCTACA ATGCCGATGT	CTAGAAGAAC GATCTTCTTG		ATCTGCGCTC TAGACGCGAG
3801		AGTTACCTTC TCAATGGAAG	GGAAAAAGAG CCTTTTTCTC		TTGATCCGGC AACTAGGCCG
3851		CCGCTGGTAG GGCGACCATC	CGGTGGTTTT GCCACCAAAA		AGCAGCAGAT TCGTCGTCTA
3901	TACGCGCAGA ATGCGCGTCT	AAAAAAGGAT TTTTTTCCTA	CTCAAGAAGA GAGTTCTTCT	TCCTTTGATC AGGAAACTAG	TTTTCTACGG AAAAGATGCC
3951	GGTCTGACGC CCAGACTGCG	TCAGTGGAAC AGTCACCTTG	GAAAACTCAC CTTTTGAGTG	GTTAAGGGAT CAATTCCCTA	TTTGGTCAGA AAACCAGTCT
4001	TCTAGCACCA AGATCGTGGT	GGCGTTTAAG CCGCAAATTC	GGCACCAATA CCGTGGTTAT	ACTGCCTTAA TGACGGAATT	AAAAATTACG TTTTTAATGC
4051		CCACTCATCG GGTGAGTAGC	CAGTACTGTT GTCATGACAA		AAGCATTCTG TTCGTAAGAC
4101		AGCCATCACA TCGGTAGTGT	AACGGCATGA TTGCCGTACT		TCGCCAGCGG AGCGGTCGCC
4151			GCGTATAATA CGCATATTAT		
4201			TTGGCTACGT AACCGATGCA		
4251			GACGAAAAAC CTGCTTTTTG		
4301			CACCGTAACA GTGGCATTGT		
4351			TCGTCGTGGT AGCAGCACCA		<del>-</del>
4401			GAAAACGGTG CTTTTGCCAC		
4451			CTTTCATTGC GAAAGTAACG		

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		2.	2143	Fig. 3 cont	
4501				AGGCCGGATA	
	GTAAGTAGTC	CGCCCGTTCT	TACACTTATT	TCCGGCCTAT	TTTGAACACG
4551	TTATTTTTCT	TTACGGTCTT	TAAAAAGGCC	GTAATATCCA	GCTGAACGGT
	AATAAAAAGA	AATGCCAGAA	ATTTTTCCGG	CATTATAGGT	CGACTTGCCA
4601	CTGGTTATAG	GTACATTGAG	CAACTGACTG	AAATGCCTCA	AAATGTTCTT
		CATGTAACTC		TTTACGGAGT	
4651	TACGATGCCA	TTGGGATATA	TCAACGGTGG	TATATCCAGT	GATTTTTTC
	ATGCTACGGT	AACCCTATAT	AGTTGCCACC	ATATAGGTCA	CTAAAAAAAG
4701	TCCATTTTAG	CTTCCTTAGC	TCCTGAAAAT	CTCGATAACT	CAAAAAATAC
	AGGTAAAATC	GAAGGAATCG	AGGACTTTTA	GAGCTATTGA	GTTTTTTATG
4751	GCCCGGTAGT	GATCTTATTT	CATTATGGTG	AAAGTTGGAA	CCTCACCCGA
	CGGGCCATCA	CTAGAATAAA	GTAATACCAC	TTTCAACCTT	GGAGTGGGCT
4801	CGTCTAATGT	GAGTTAGCTC	ACTCATTAGG	CACCCCAGGC	TTTACACTTT
	GCAGATTACA	CTCAATCGAG	TGAGTAATCC	GTGGGGTCCG	AAATGTGAAA
4851	ATGCTTCCGG	CTCGTATGTT	GTGTGGAATT	GTGAGCGGAT	AACAATTTCA
	TACGAAGGCC	GAGCATACAA	CACACCTTAA	CACTCGCCTA	TTGTTAAAGT
	M13 Reverse	e primer 10 ======	00.0%	XbaI ~~~~~	
4901			ATGATTACGA	ATTTCTAGAT	AACGAGGGCA
				TAAAGATCTA	
4951	AAAAATGAAA	AAGACAGCTA	TCGCGATTGC	AGTGGCACTG	GCTGGTTTCG
	TTTTTACTTT	TTCTGTCGAT	AGCGCTAACG	TCACCGTGAC	CGACCAAAGC
		EcoRV			
		~~~			
5001		GCAGGCCGAT			
	GATGGCATCG	CGTCCGGCTA			

23/43

Fig. 4a

	Framework 1	CDR 1	Framework 2	CDR 2
Position	1 2 2 4 5 6 7 8 9 0 1 1 2 2 4 5 6 7 8 9 0 1 1 2 2	3 3 4 4 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6	4 4 6 6 6 7 7 7 6 6 7 7 7 7 7 7 7 7 7 7	7. C C C C C C C C C C C C C C C C C C C
	BanII		KpnI SexAI	Asel
MS-Roche #3	D I V L T Q S P A T L S L S P G E R A T L S C	S S S V S O S A R	0	L I Y G A S S R A
MS-Roche #7	DIVLTQSPATLSLSPGERATLSC	R-ASQSVSSS	Y L A W Y Q Q K P G Q A P R	LLIYGASSR
MS-Roche #8	DIVLTQSPATLSLSPGERATLSCR	ASQSVSS	Y L A W Y Q Q K P G Q A P R	LLIYGASSR
	Framework 1	CDR 1	Framework 2	CDR 2
Position	1 2	E	4	ហ
	12345678901234567890123	5 6 7 8 9 0 1 a b 2 3 4 5	2 3 4	0 1 2 a b c 3 4 5
	Mtel	BspEI	BstXI XhoI	
MS-Roche #3	QVQLVESGGGLVQPGGSLRLSCAA	S G F T F S S Y A M S	W V R Q A P G K G L E W V S	A I S G S G G
MS-Roche #7	Q V Q L V E S G G G L V Q P G G S L R L S C A A	SGFTFSST YAMS	W V R Q A P G K G L E W V S	A I S G S G G
MS-Roche #8	O V Q L V E S G G G L V Q P G G S L R L S C A	ASGETESS - YAMS	W V R O A P G K G L E W V S	8 I S G C . S G G

Sequence of MS-Roche#3, #7 and #8

24/43

Fig. 4a cont.

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Fig. 4b

Sequence of MS-Roche#3, #7 and #8

_    -	Framework 1	CDR 1
Position	1 2 3 4 5 6 7 8 9 0 EcoRV BanII	4 5 6 7 8 9 0 a b c d e f 1 2 3 4 5 6 Kpnl
MS-Roche #3 MS-Roche #7 MS-Roche #8	#3 GAT ATC GTG CTG ACC CAG AGC CTG ACC CTG AGC CTG TCT CCG GGC GAA CGT GCG ACC CTG AGC TGC AGA GGC GAG GGC GTG AGC GTG	AGA GCG AGC CAG AGC GTG AGC AGC — — — — — — AGC TAT CTG GCG TGG TAC AGA GCG AGC CAG AGC GTG AGC AGC — — — — — — AGC TAT CTG GCG TGG TAC AGA GCG AGC CAG AGC GTG AGC AGC — — — — AGC TAT CTG GCG TGG TAC
H	Framework 1	CDR 1.
Position	n 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 Med	4 5 6 7 8 9 0 1 a b 2 3 4 5 6 7 8 9 0 BebXI
MS-Roche #3 MS-Roche #7 MS-Roche #8	CAG GTG CAA TTG GTG GAA AGC GGC GGC CTG GTG CAA CCG GGC GGC AGC CTG CGT CTG CAG GTG CAA TTG GTG GAA AGC GGC GGC GGC CTG GTG CAA CCG GGC GGC AGC CTG CTG CAG GTG CAA TTG GTG GAA AGC GGC GGC GGC CTG GTG CAA CCG GGC GGC AGC CTG CTG CAG GTG CAA TTG GTG GAA AGC GGC GGC GGC CTG GTG CAA CCG GGC GGC AGC CTG CGT CTG	AGC TGC GCG GCC TCC GGA TTT ACC TTT AGC AGC TAT GCG ATG AGC TGG GTG CGC CAA GCC AGC TGC GCG GCC TCC GGA TTT ACC TTT AGC AGC TAT GCG ATG AGC TGG GTG CGC CAA GCC AGC TGC GCG CCC TCC GGA TTT ACC TTT AGC AGC TAT GCG ATG AGC TGG GTG CGC CAA GCC

25/43

Fig. 4b cont.

Framework 2	CDR 2		Framework 3	
4	5	9	7	
8 9 0 1 2 3 4 5 6 7 8	9 0 1 2 3 4 5 6	7 8 9 0 1 2 3 4	8 9 0 1 2 3 4 5 6 7 8 9 0 1	1 2 Ph.7
SexAI  SanDI  SanDI  SanDI  SanDI  AGG CAG AGC CGT GCA ACT GGG GTC CCG  AGG CAG AGC CGT GCA ACT GGG GTC CCG  AGG CAG AGA CCA GGT CAA GCA CCG CGT CTA TTA ATT TAT GGC GCG AGC CGT GCA ACT GGG GTC CCG  AGG CAG AAA CCA GGT CAA GCA CCG CGT CTA TTA ATT TAT GGC GCG AGC CGT GCA ACT GGG GTC CCG  AGG CAG AAA CCA GGT CAA GCA CCG CCT CTA TTA ATT TAT GGC GCG AGC CGT GCA ACT GGG GTC CCG	TAT GGC GCG AGC AGC CGT GCA ACT C TAT GGC GCG AGC AGC CGT GCA ACT C TAT GGC GCG AGC AGC CGT GCA ACT C	SanDi GGG GTC CCG GCG CGT TTT AGC GGC T GGG GTC CCG GCG CGT TTT AGC GGC T GGG GTC CCG GCG CGT TTT AGC GGC T	GCG CGT TITT AGC GGC TCT GGA TCC GGC ACG GAT TITT ACC CTG ACC ATT AGC AGC CTG GAA CCT GAA GAC GCG CGT TITT AGC GGC TCT GGA TCC GGC ACG GAT TITT ACC CTG ACC ATT AGC AGC CTG GAA CCT GAA GAC GCC CTT GGA TCC GGC ACG GAT TITT ACC CTG ACC ATT AGC AGC CTG GAA CCT GAA GAC GCC CTT AGC GGC TCT GGA CCT GAA GAC GCC ACG GAT TTT ACC CTG ACC ATT AGC AGC CTG GAA CCT GAA GAC	AA GAC AA GAC AA GAC
Framework 2	CDR2		Framework 3	work 3
ro.		9	7	
2 3 4 5 6 7 8 9 0 1 2 XhoI	a b c 3 4 5 6 7	8 9 0 1 2 3 4 5	6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 BstEII	2 a
CCT GGG AAG GGT CTC GAG TGG GTG AGC GCG ATT AGC GGT CCT GGG AAG GGT CTC GAG TGG GTG AGC GCG ATT AGC GGT CCT GGG AAG GGT CTC GAG TGG GTG AGC GCG ATT AGC GGT CTC GGG AAG GGT CTC GAG TGG GTG AGC GCG ATT AGC GGT	- AGC GGC GGC AGC ACC - AGC GGC GGC AGC ACC - AGC GGC GGC AGC ACC ACC ACC ACC ACC ACC	SC GGC GGC AGC ACC TAT TAT GGG GAT AGC GTG AAA GGC GGT TIT ACC ATT TCA CGT GGC GGC AGC AGC ACC TAT TAT GGG GAT AGC GTG AAA GGC GGT TIT ACC ATT TCA CGT GG GGC GGC AGC ACC ACC TAT TAT GGG GAT AGC GTG AAA GGC CGT TIT ACC ATT TCA CGT GGC GGC AGC AGC ACC ACC ATT TCA CGT	SET TIT ACC ATT TCA CGT GAT APT TCG APA AAC ACC CTG TAT CTG CAA ATG SGT TIT ACC ATT TCA CGT GAT APT TCG APA AAC ACC CTG TAT CTG CAA ATG SGT TIT ACC ATT TCA CGT GAT APT TCG APA AAC ACC CTG TAT CTG CAA ATG	TG AAC TG AAC TG AAC

Fig. 4b cont.

										Framework 4	11	3 4 5 6 7 8 9 0 1 2	StyI		тт сат стт тес сес саа сес стс стс стс втс все стт де	тт ват втг тве вес сла вес дс сте вте дсе втт д	TIT GAT GIT TGG GGC CAA GGC ACC CTG GTG ACG GTT AG
Framework 4	10	a b 6 7 8 9 0 1 2 3 4 5 6 7 8 9	MscI		GTT ACC TIT GGC CAG GGT ACG AAA GTT GAA ATT AAA CGT ACG	TIT ACC TIT GGC CAG GGT ACG AAA GTT GAA ATT AAA CGT ACG	CCT ACC TIT GGC CAG GGT ACG AAA GTT GAA ATT AAA CGT ACG			CDR3	10	1567890 a b c d e f g h i j 12		の の の の の の の の の の の の の の の の の の の	TIGC GCG GET CIT ACT CAT TAT GCT CGT TAT TAT CGT TAT	CGT TAT	
CDR 3	6	6 7 8 9 0 1 2 3 4 5 a		1	TAT TAT TGC CAG CAG GTT TAT AAT CCT CCT	TAT TAT TGC TTT CAG CTT TAT TCT GAT CCT	TAT TAT TGC CAG CAT TCT TCT TTT CCT	The state of the s			6	4 5 6 7 8 9 0 1 2 3 4	Eagl		GCG GAA GAT ACG GCC GTG TAT TAT	GCG GAA GAT ACG GCC GTG TAT	GCG GAA GAT ACG GCC GTG TAT
		3 4 5			गम ६८६ हम	TTT GCG ACT	TTT GCG ACT					b с 3			AGC CTG CGT	AGC CTG CGT	AGC CTG CGT

28/43 Fig. 5

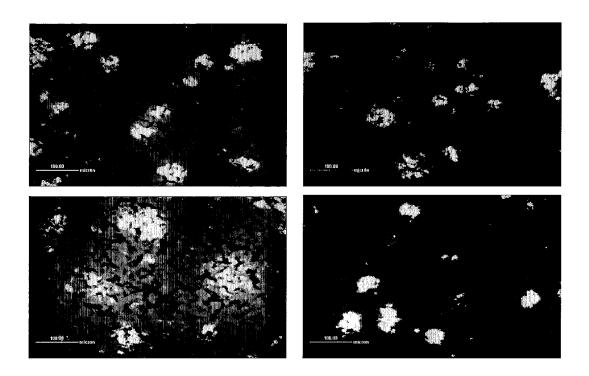
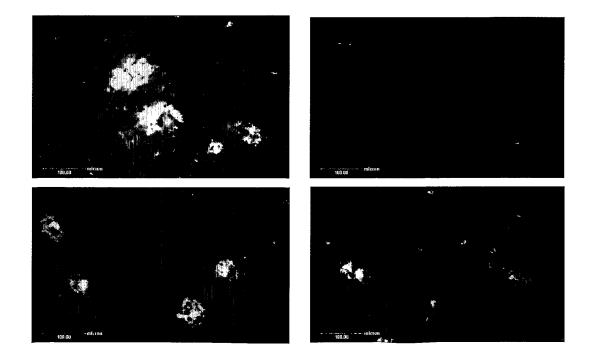
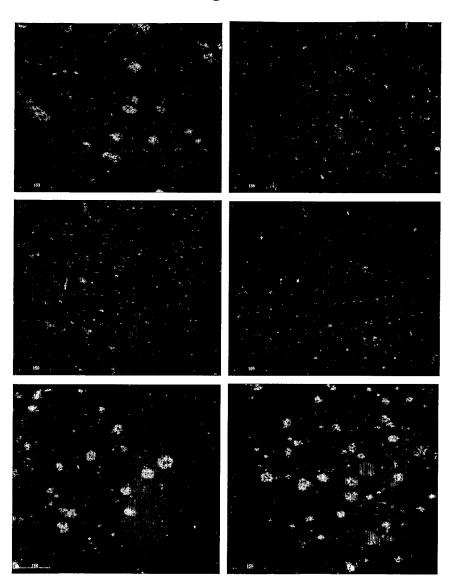


Fig. 6

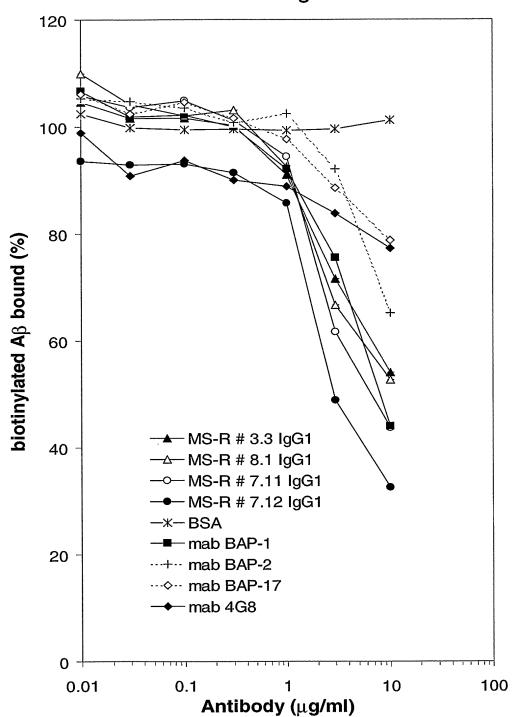


29/43 Fig. 7

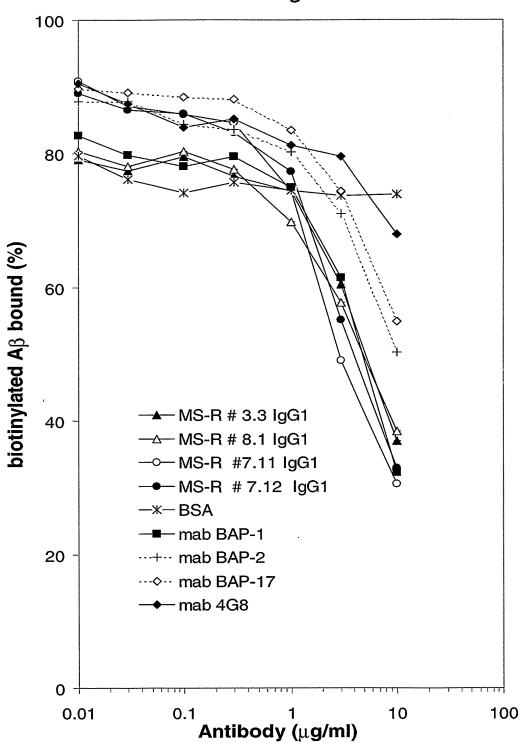


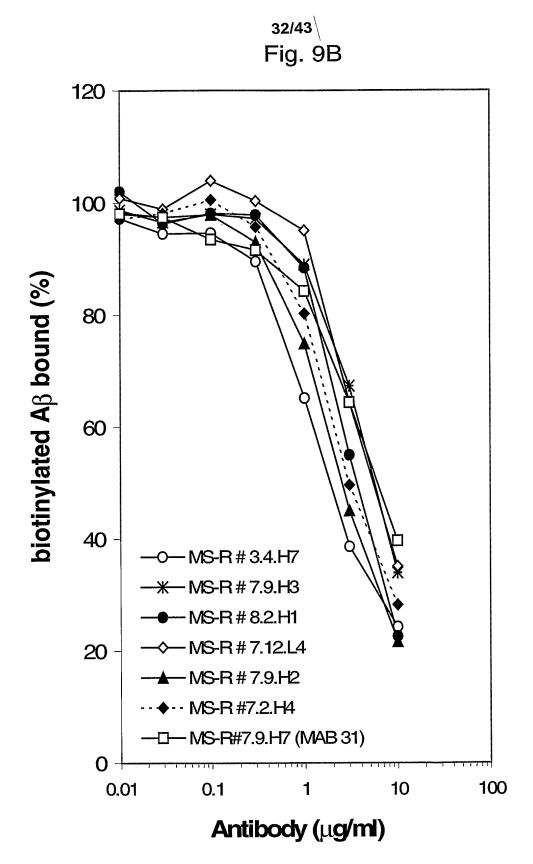
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Fig. 8



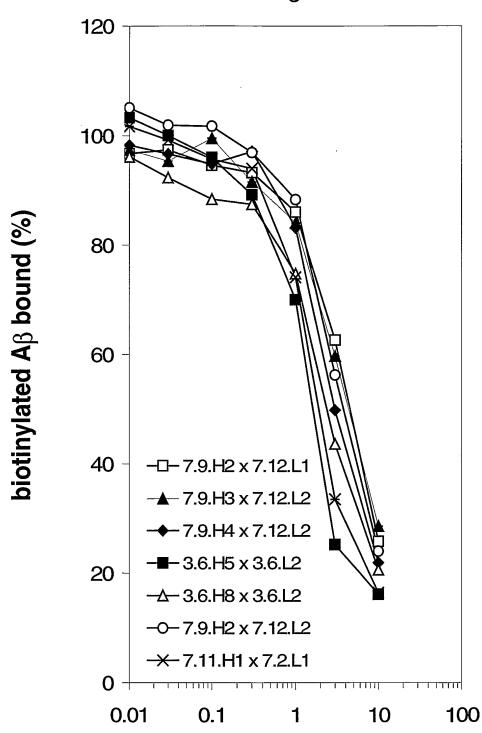




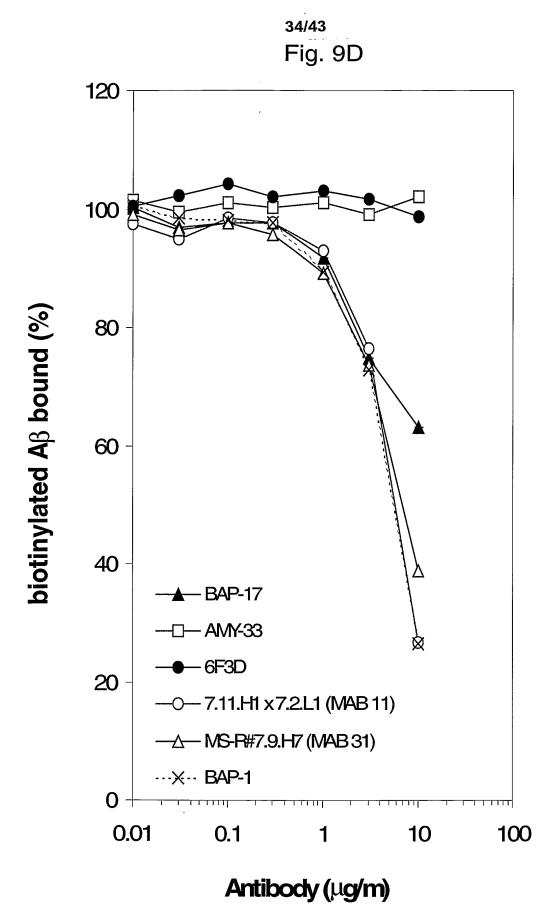


33/43

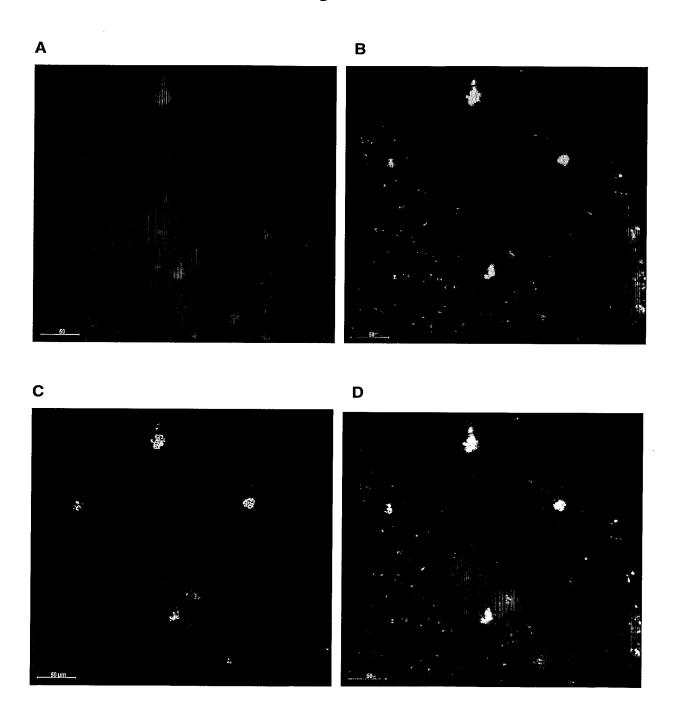
Fig. 9C



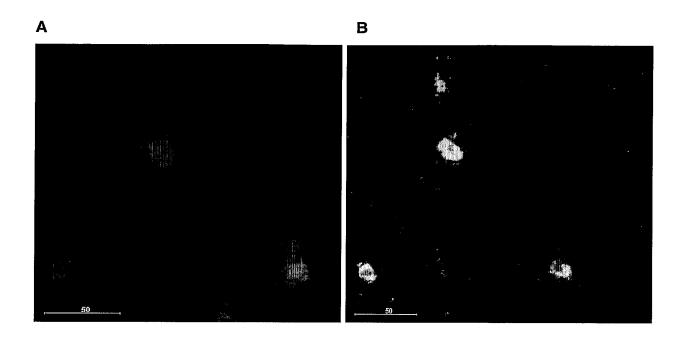
Antibody (µg/ml)

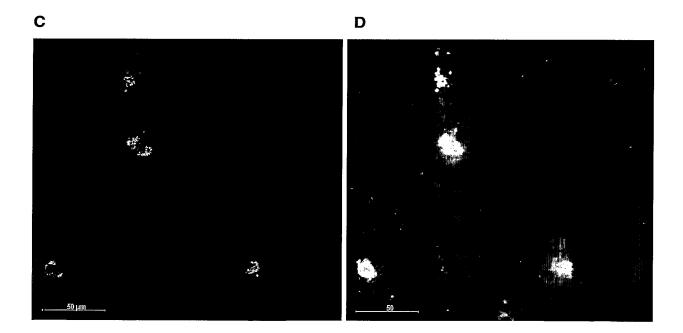


35/43 \ Fig. 10

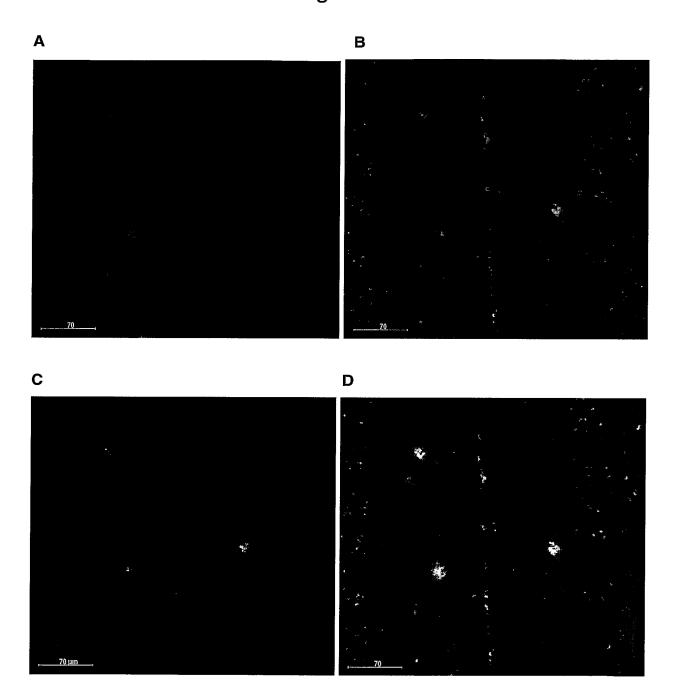


36/43 Fig. 11

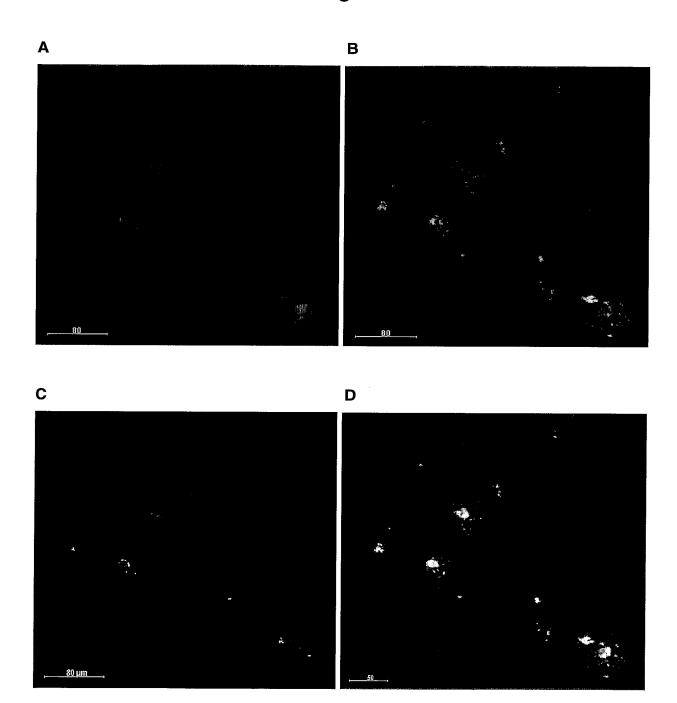




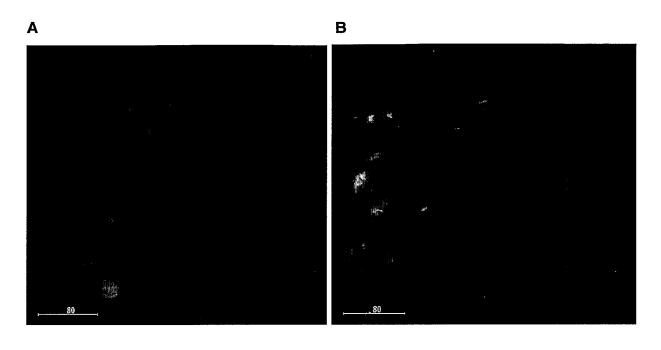
37/43 Fig. 12

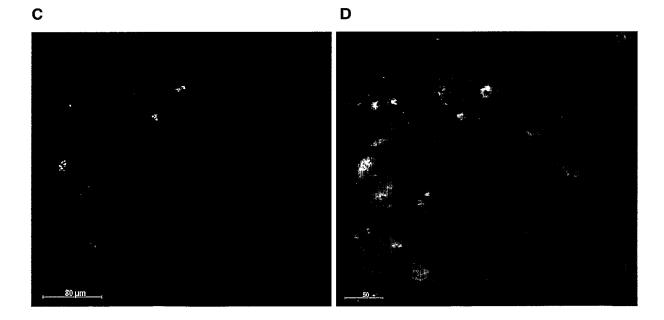


<sup>38/43</sup> Fig. 13



39/43/ Fig. 14





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Fig. 15 - 1

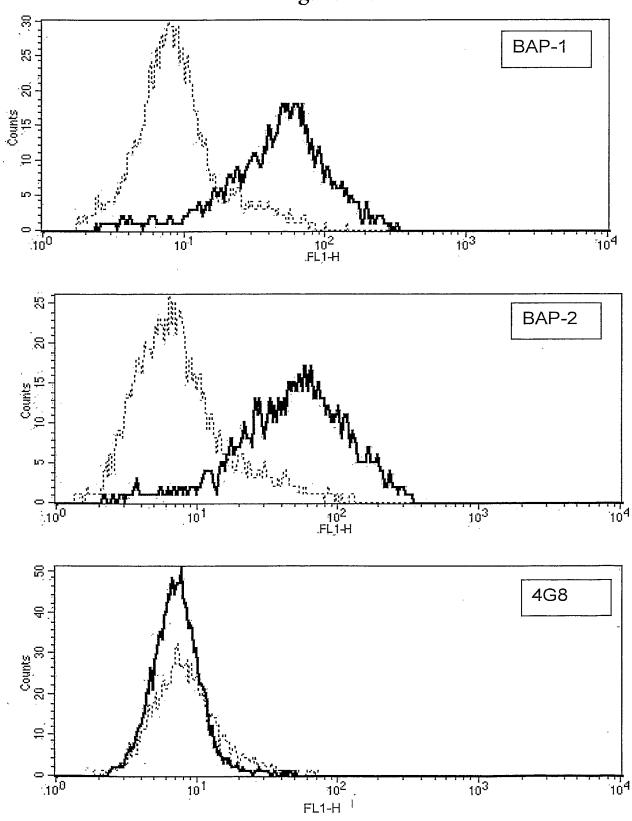




Fig. 15 - 2

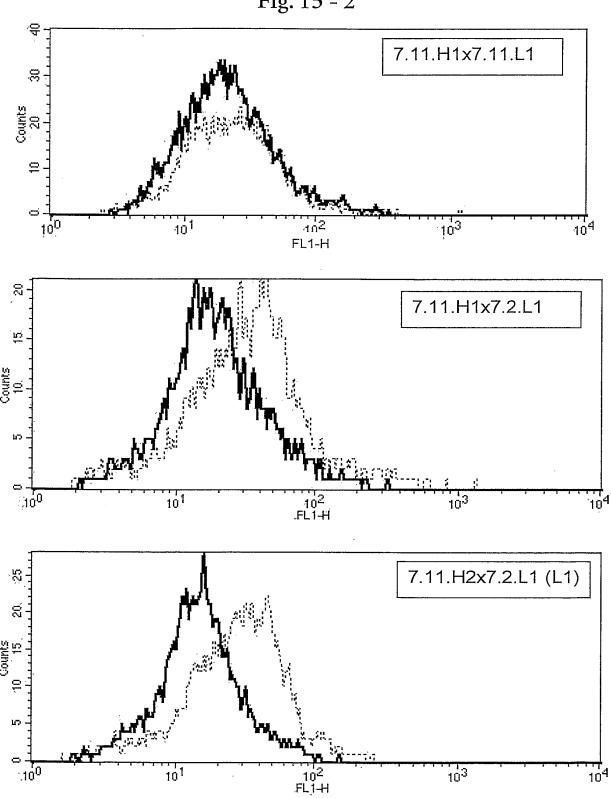


Fig. 15 - 3

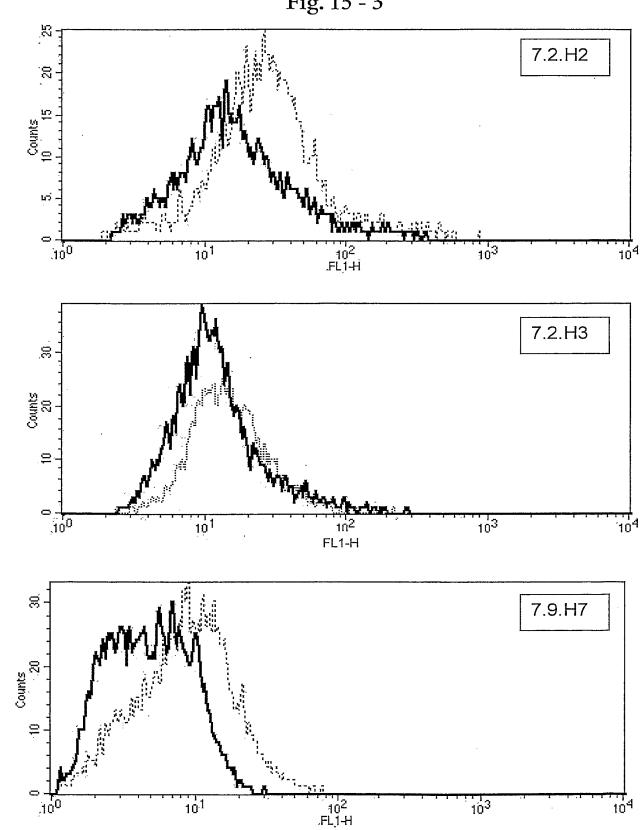
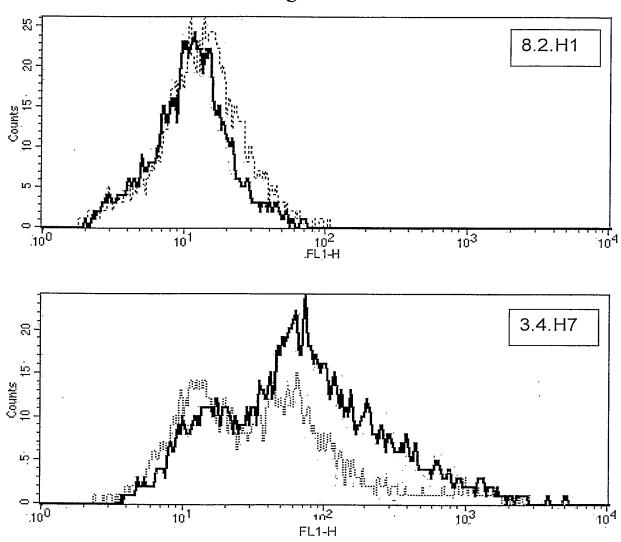


Fig. 15 - 4



#### SEQUENCE LISTING

<110> F. Hoffmann-La Roche AG MorphoSys AG <120> Anti A-beta antibodies and their use <130> F 2842 PCT <140> EP 02003844.4 <141> 2002-02-20 <150> EP 02003844.4 <151> 2002-02-20 <160> 414 <170> PatentIn version 3.1 <210> 1 <211> 9 <212> PRT <213> artificial sequence <220> <223> synthetic construct; first region of beta-A4 peptide <400> 1 Ala Glu Phe Arg His Asp Ser Gly Tyr <210> 2 <211> 14 <212> PRT <213> artificial sequence <220> <223> synthetic construct; second region of beta-A4 peptide <400> 2 Val His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly <210> 3 <211> 368 <212> DNA <213> artificial sequence <220> <223> synthetic construct; VH-region of MS-Roche#3 <400> 3 caggtgcaat tggtggaaag cggcggcggc ctggtgcaac cgggcggcag cctgcgtctg 60 agctgcgcgg cctccggatt tacctttagc agctatgcga tgagctgggt gcgccaagcc

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gcggatagcg tgaaaggccg ttttaccatt tcacgtgata attcgaaaaa caccctgtat	240
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1 5 10 15	
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Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val 35 40 45	
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val 50 55 60	
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr 65 70 75 80	
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys 85 90 95	
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# WO 03/070760 PCT/EP03/01759 3/165

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120

180

240

300

360

379

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120

115

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tctcg	gtgg	tt a	taat	ggtt	a tt	atca	taag	, ttt	gatg	rttt	gggg	ccaa	igg c	cacco	tggt	g	360
acggt	tag	ct c	agc														374
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1				5			-	_	10					15	_		
Ser I	Leu	Arg	Leu 20	Ser	Cys	Ala	Ala	Ser 25	Gly	Phe	Thr	Phe	Ser 30	Ser	Tyr		
Ala N	Met	Ser 35	Trp	Val	Arg	Gln	Ala 40	Pro	Gly	Lys	Gly	Leu 45	Glu	Trp	Val		
Ser Æ	Ala 50	Ile	Ser	Gly	Ser	Gly 55	Gly	Ser	Thr	Tyr	Tyr 60	Ala	Asp	Ser	Val		
Lys ( 65	Gly	Arg	Phe	Thr	Ile 70	Ser	Arg	Asp	Asn	Ser 75	Lys	Asn	Thr	Leu	Tyr 80		
Leu (	Gln	Met	Asn	Ser 85	Leu	Arg	Ala	Glu	Asp 90	Thr	Ala	Val	Tyr	Tyr 95	Cys		

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<pre>&lt;211&gt; 110 &lt;212&gt; PRT &lt;213&gt; artificial sequence  &lt;220&gt; &lt;223&gt; synthetic construct; VL-region of MS-Roche#3  &lt;400&gt; 10  Asp Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly 1</pre>	

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Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Val Pro Ala Arg Phe Ser

50

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                                                                       24
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Val
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aagggtctcg agtgggtgag cgctatttct gagtctggta agactaagta ttatgctgat	180
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Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu 1 15	
a m	
1 5 10 15  Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met	
1 5 10 15  Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met 20 25 30  Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ala	
10 15  Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met 25 30  Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ala 40  Ile Ser Glu Ser Gly Lys Thr Lys Tyr Tyr Ala Asp Ser Val Lys Gly	

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Ile Ser Glu Tyr Ser Lys Phe Lys Tyr Tyr Ala Asp Ser Val Lys Gly 50 55 60

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Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ala

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40 45 35 Ile Asn Tyr Asn Gly Ala Arg Ile Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln 75 70 Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Lys Gly Asn Thr His Lys Pro Tyr Gly Tyr Val Arg Tyr Phe Asp 110 100 105 Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser 115 120 <210> 38 <211> 372 <212> DNA <213> artificial sequence <220> <223> synthetic construct; VH MS-Roche#7.9H2 x 7.12L2 <400> 38 60 caattqqtqq aaagcqqcqq cggcctggtg caaccgggcg gcagcctgcg tctgagctgc 120 qcqqcctccq qatttacctt tagcagctat gcgatgagct gggtgcgcca agcccctggg 180 aaqqqtctcq aqtqqqtqaq cqctattaat gctgatggta atcgtaagta ttatgctgat tctgttaagg gtcgttttac catttcacgt gataattcga aaaacaccct gtatctgcaa 240 atgaacaqcc tgcgtgcgga agatacggcc gtgtattatt gcgcgcgtgg taagggtaat 300 actcataaqc cttatqqtta tqttcqttat tttgatqttt ggggccaagg caccctggtg 360 372 acggttagct ca <210> 39 <211> 124 <212> PRT <213> artificial sequence <220> <223> synthetic construct; VH MS-Roche#7.9H2 x 7.12L2 <400> 39 Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu

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Ile Asn Ala 50	Asp Gly	Asn Arg Ly 55	ys Tyr T	Tyr Ala	Asp Ser 60	Val Lys	Gly
Arg Phe Thr 65		Arg Asp A 70	sn Ser L	Lys Asn 75	Thr Leu	Tyr Leu	Gln 80
Met Asn Ser	Leu Arg 2 85	Ala Glu A		Ala Val 90	Tyr Tyr	Cys Ala 95	Arg
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tctgttaagg	gtcgtttta	c catttca	cgt gata	aattcga	aaaacacc	cct gtat	ctgcaa 240
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Arg Leu	Ser	Cys 20	Ala	Ala	Ser	Gly	Phe 25	Thr	Phe	Ser	Ser	Tyr 30	Ala	Met		
Ser Trp	Val 35	Arg	Gln	Ala	Pro	Gly 40	Lys	Gly	Leu	Glu	Trp 45	Val	Ser	Ala		
Ile Asn 50	Ala	Val	Gly	Met	Lys 55	Lys	Phe	Tyr	Ala	Asp 60	Ser	Val	Lys	Gly		
Arg Phe 65	Thr	Ile	Ser	Arg 70	Asp	Asn	Ser	Lys	Asn 75	Thr	Leu	Tyr	Leu	Gln 80		
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Gly Lys	Gly	Asn 100	Thr	His	Lys	Pro	Tyr 105	Gly	Tyr	Val	Arg	Tyr 110	Phe	Asp		
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Ser Tr	p Val 35	Arg	Gln	Ala	Pro	Gly 40	Lys	Gly	Leu	Glu	Trp 45	Val	Ser	Gly	
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Arg Ph 65	e Thr	Ile	Ser	Arg 70	Asp	Asn	Ser	Lys	Asn 75	Thr	Leu	Tyr	Leu	Gln 80	
Met As	n Ser	Leu	Arg 85	Ala	Glu	Asp	Thr	Ala 90	Val	Tyr	Tyr	Cys	Ala 95	Arg	
Gly Ly	s Gly	Asn 100	Thr	His	Lys	Pro	Tyr 105	Gly	Tyr	Val	Arg	Tyr 110	Phe	Asp	
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Ser Trp Val Arg Gln Ala Pro Gl		Glu Trp Val Se 45	r Gly
Ile Asn Ala Ala Gly Phe Arg Th	hr Tyr Tyr Ala	Asp Ser Val Ly	s Gly
Arg Phe Thr Ile Ser Arg Asp As 65 70	sn Ser Lys Asn 75	Thr Leu Tyr Le	u Gln 80
Met Asn Ser Leu Arg Ala Glu As 85	sp Thr Ala Val 90	Tyr Tyr Cys Al 95	
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Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu 35 40 45	
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Val Pro Ala Arg Phe Ser 50 55 60	
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu 65 70 75 80	
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Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu 65 70 75 80	
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Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu 35 40 45											
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Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu 65 70 75 80											
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Tyr	Leu	Ala 35	Trp	Tyr	Gln	Gln	Lys 40	Pro	Gly	Gln	Ala	Pro 45	Arg	Leu	Leu	
Ile	Ser 50	Gly	Ser	Ser	Asn	Arg 55	Ala	Thr	Gly	Val	Pro 60	Ala	Arg	Phe	Ser	
Gly 65	Ser	Gly	Ser	Gly	Thr 70	Asp	Phe	Thr	Leu	Thr 75	Ile	Ser	Ser	Leu	Glu 80	
Pro	Glu	Asp	Phe	Ala 85	Val	Tyr	Tyr	Cys	Leu 90	Gln	Leu	Tyr	Asn	Ile 95	Pro	
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Tyr Le	u Ala 35	Trp	Tyr	Gln	Gln	Lys 40	Pro	Gly	Gln	Ala	Pro 45	Arg	Leu	Leu		
Ile Ty	r Gly	Ala	Ser	Ser	Arg 55	Ala	Thr	Gly	Val	Pro 60	Ala	Arg	Phe	Ser		
Gly Se	r Gly	ser Ser	Gly	Thr 70	Asp	Phe	Thr	Leu	Thr 75	Ile	Ser	Ser	Leu	Glu 80		
Pro Gl	u Asp	Phe	Ala 85	Thr	Tyr	Tyr	Cys	Gln 90	Gln	Val	Tyr	Ser	Pro 95	Pro		
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Pro	Glu	Asp	Phe	Ala 85	Thr	Tyr	Tyr	Cys	Gln 90	Gln	Ile	Tyr	Ser	Phe 95	Pro	
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Asn Ile Ser Gly Ser Gly Ser Ser Thr Tyr Tyr Ala Asp Ser Val Lys
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15 5 10 1 Gly <210> 165 <211> 17 <212> PRT <213> artificial sequence <220> <223> synthetic construct; HCDR3 of MS-Roche#7.12 <400> 165 Gly Lys Gly Asn Thr His Lys Pro Tyr Gly Tyr Val Arg Tyr Phe Asp 10 Val <210> 166 <211> 8 <212> PRT <213> artificial sequence <220> <223> synthetic construct; LCDR3 of MS-Roche#7.13 <400> 166 His Gln Val Tyr Ser Pro Pro Phe <210> 167 <211> 17 <212> PRT <213> artificial sequence <220> <223> synthetic construct; HCDR2 of MS-Roche#7.2H1 <400> 167 Ala Ile Asn Ala Asn Gly Leu Lys Lys Tyr Tyr Ala Asp Ser Val Lys 10 Gly <210> 168 <211> 17

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His Gln Val Tyr Ser His Pro Phe

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Gly

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<211> 17
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<210> 189
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Gly

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<210> 207

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<210> 215
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Gly
<210> 220
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Gly

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<220>
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<220> <221> <222> <223>	MISC_ (85). Xaa =	(85	5)	Thr	or W	al"										
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Asp Il 1	e Gln	Met	Thr 5	Gln	Ser	Pro	Ser	Ser 10	Leu	Ser	Ala	Ser	Val 15	Gly		
Asp Ar	g Val	Thr 20	Ile		Cys			Ser				Ser 30	Ser	Tyr		
Leu Al	a Trp 35	Tyr	Gln	Gln	Lys	Pro 40	Gly	Lys	Ala	Pro	Lys 45	Leu	Leu	Ile		
Tyr Al		Ser	Ser	Leu	Gln 55	Ser	Gly	Val	Pro	Ser 60	Arg	Phe	Ser	Gly		
Ser Gl 65	y Ser.	Gly	Thr	Asp 70	Phe	Thr	Leu	Thr	Ile 75	Ser	Ser	Leu	Gln	Pro 80		
Glu As	sp Phe	Ala	Xaa 85	Tyr	Tyr	Cys	Xaa	Gln 90	Xaa	Xaa	Xaa	Xaa	Xaa 95	Xaa		

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr 105 100 <210> 226 <211> 114 <212> PRT <213> artificial sequence <220> <223> synthetic construct; VL kappa2 <220> <221> misc\_feature <222> (101)..(101)<223> Xaa = any amino acid of a mixture of Ala, Asp, Glu, Phe, Gly, His , Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, or Tyr <220> <221> misc\_feature <222> (94)...(94)<223> Xaa = any amino acid of a mixture of Phe, His, Ile, Leu, Met or G <220> <221> misc\_feature <222> (96)..(96) <223> Xaa = any amino acid of a mixture of Ala, Asp, Glu, Phe, Gly, His , Ile, Lys, Leu, Met, Asn, Gln, Arg, Ser, Thr, Val, Trp or Tyr <220> <221> misc\_feature <222> (97)..(97) <223> Xaa = any amino acid of a mixture of Asp, Gly, Asn, Ser or Tyr <220> <221> misc\_feature <222> (98)..(98) <223> Xaa = any amino acid of a mixture of Ala, Asp, Gly, His, Leu, Asn or Ser <220> <221> misc feature (99)..(99) <222> <223> Xaa = any amino acid of a mixture of Ala, Asp, Glu, Phe, Gly, His , Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, or Tyr <220> <221> misc\_feature

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Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser 20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser 35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro 50 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile 65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Xaa Gln Xaa 85 90 95

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                                25
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Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
        35
                             40
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Val Pro Ala Arg Phe Ser
    50
                        55
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Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu
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Pro Glu Asp Phe Ala Xaa Tyr Tyr Cys Xaa Gln Xaa Xaa Xaa Xaa
Xaa Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
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<223> Xaa = any amino acid of a mixture of Ala, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, or Tyr

<220>

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<223> Xaa = any amino acid of a mixture of Leu, Pro or Ser

<400> 228

Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly 1  $\phantom{\bigg|}$  5  $\phantom{\bigg|}$  10  $\phantom{\bigg|}$  15

Glu Arg Ala Thr Ile Asn Cys Arg Ser Ser Gln Ser Val Leu Tyr Ser 20 25 30

Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln 35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val 50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr 65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Xaa Gln 85 90 95

Xaa Xaa Xaa Xaa Xaa Thr Phe Gly Gln Gly Thr Lys Val Glu Ile  $100 \hspace{1.5cm} 105 \hspace{1.5cm} 110$ 

Lys Arg Thr 115

<210> 229

<211> 111

<212> PRT

<213> artificial sequence

<220>

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<220>

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<223> Xaa = any amino acid except a Cys or a deletion
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<222> (94)..(96)
<223> Xaa = any amino acid except a Cys
<220>
<221> MISC_FEATURE
<222> (92)..(92)
<223> Xaa = any amino acid of Cys, Phe, His, Arg, Trp or Tyr
<400> 229
Asp Ile Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
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            2.0
Tyr Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35
Ile Tyr Asp Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
                        55
    50
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
65
                    70
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Xaa Asp Xaa Xaa
                85
Xaa Xaa Xaa Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100
                                105
<210> 230
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<221> MISC FEATURE
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<220> <221> <222> <223>	MISC (93) Xaa	(93	3)	.no a	acid	of (	Cys,	Phe,	His	s, Ai	g, 1	Prp (	or Ty	/r
<220> <221> <222> <223>	MISC (95) Xaa =	(97	7)	ino a	acid	exce	ept a	а Суз	5					
<220> <221> MISC_FEATURE <222> (98)(99) <223> Xaa = any amino acid except a Cys or a deletion														
<400>	230													
Asp Il 1	e Ala	Leu	Thr 5	Gln	Pro	Ala	Ser	Val 10	Ser	Gly	Ser	Pro	Gly 15	Gln
Ser Il	e Thr	Ile 20	Ser	Cys	Thr	Gly	Thr 25	Ser	Ser	Asp	Val	Gly 30	Gly	Tyr
Asn Ty	r Val 35	Ser	Trp	Tyr	Gln	Gln 40	His	Pro	Gly	Lys	Ala 45	Pro	Lys	Leu
Met Il 50	e Tyr	Asp	Val	Ser	Asn 55	Arg	Pro	Ser	Gly	Val 60	Ser	Asn	Arg	Phe
Ser Gl 65	y Ser	Lys	Ser	Gly 70	Asn	Thr	Ala	Ser	Leu 75	Thr	Ile	Ser	Gly	Leu 80
Gln Al	a Glu	Asp	Glu 85	Ala	Asp	Tyr	Tyr	Cys 90	Gln	Ser	Xaa	Asp	Xaa 95	Xaa
Xaa Xa	a Xaa	Xaa 100	Val	Phe	Gly	Gly	Gly 105	Thr	Lys	Leu	Thr	Val 110	Leu	Gly
<210><211><211><212><213>	<211> 109 <212> PRT													
<220> <223>	synt	heti	c co	nstr	uct;	VL :	lambo	da3						

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<223> Xaa = any amino acid of Cys, Phe, His, Arg, Trp or Tyr
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       (92)..(94)
<223> Xaa = any amino acid except a Cys
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<222> (95)..(96)
<223> Xaa = any amino acid except a Cys or a deletion
<400> 231
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Thr Ala Arg Ile Ser Cys Ser Gly Asp Ala Leu Gly Asp Lys Tyr Ala
            20
Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
        35
Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Glu
                                                             80
65
Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Xaa Asp Xaa Xaa Xaa Xaa
                85
Xaa Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100
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<211> 127
<212> PRT
<213> artificial sequence
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<220> <223> synthetic construct; VH1A <220> <221> MISC\_FEATURE <222> (99)..(112) <223> Xaa = any amino acid or a deletion <220> <221> MISC FEATURE <222> (116)..(116) <223> Xaa = any amino acid out of a mixture of Phe, His, Ile, Leu, Asn, Pro, Ser, Val, Trp or Tyr <220> <221> MISC FEATURE <222> (114)..(114)<223> Xaa = any amino acid out of a mixture of Ala, Asp, Glu, Phe, Gly, Ile, Leu, Met, Pro, Gln, Ser, Thr, Val or Tyr <220> <221> MISC\_FEATURE <222> (113)..(113) <223> Xaa = any amino acid <400> 232 Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys 100 105

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Xaa Xaa Asp Xaa Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser 120 <210> 233 <211> 127 <212> PRT <213> artificial sequence <220> <223> synthetic construct; VH1B <220> <221> MISC\_FEATURE <222> (99)..(112) <223> Xaa = any amino acid or a deletion <220> <221> MISC\_FEATURE <222> (113)..(113) <223> Xaa = any amino acid <220> <221> MISC\_FEATURE  $\langle 222 \rangle$  (114)...(114)<223> Xaa = any amino acid out of a mixture of Ala, Asp, Glu, Phe, Gly, Ile, Leu, Met, Pro, Gln, Ser, Thr, Val or Tyr <220> <221> MISC\_FEATURE <222> (116)..(116)<223> Xaa = any amino acid out of a mixture of Phe, His, Ile, Leu, Asn, Pro, Ser, Val, Trp or Tyr <400> 233 Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr 25 20 Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met 35 40 Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe 50 55 Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr 75 70 65

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Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys 90 95 105 Xaa Xaa Asp Xaa Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser 120 125 <210> 234 <211> 128 <212> PRT <213> artificial sequence <220> <223> synthetic construct; VH2 <220> <221> MISC\_FEATURE <222> (100)..(113) <223> Xaa = any amino acid or a deletion <220> <221> MISC\_FEATURE <222> (114)..(114) <223> Xaa = any amino acid <220> <221> MISC\_FEATURE <222> (117)..(117) <223> Xaa = any amino acid out of a mixture of Phe, His, Ile, Leu, Asn, Pro, Ser, Val, Trp or Tyr <220> <221> MISC FEATURE <222> (115)..(115) <223> Xaa = any amino acid out of a mixture of Ala, Asp, Glu, Phe, Gly, Ile, Leu, Met, Pro, Gln, Ser, Thr, Val or Tyr <400> 234 Gln Val Gln Leu Lys Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser

Gly Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu

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Trp Leu Ala Leu Ile Asp Trp Asp Asp Asp Lys Tyr Tyr Ser Thr Ser 55 Leu Lys Thr Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val 70 Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr 90 85 100 105 Xaa Xaa Xaa Asp Xaa Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser 115 120 125 <210> 235 <211> 127 <212> PRT <213> artificial sequence <220> <223> synthetic construct; VH3 <220> <221> MISC\_FEATURE <222> (99)..(112)<223> Xaa = any amino acid or a deletion <220> <221> MISC\_FEATURE <222> (113)..(113) <223> Xaa = any amino acid <220> <221> MISC\_FEATURE <222> (116)..(116) <223> Xaa = any amino acid out of a mixture of Phe, His, Ile, Leu, Asn, Pro, Ser, Val, Trp or Tyr <220> <221> MISC\_FEATURE <222> (114)..(114) <223> Xaa = any amino acid out of a mixture of Ala, Asp, Glu, Phe, Gly, Ile, Leu, Met, Pro, Gln, Ser, Thr, Val or Tyr <400> 235

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly

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10 15 5 1 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr 20 25 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ala Ile Ser Gly Ser Gly Ser Thr Tyr Tyr Ala Asp Ser Val 55 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr 65 70 75 80 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys 90 100 105 Xaa Xaa Asp Xaa Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser 125 120 <210> 236 <211> 126 <212> PRT <213> artificial sequence <220> <223> synthetic construct; VH4 <220> <221> MISC\_FEATURE <222> (98)..(111) <223> Xaa = any amino acid or a deletion <220> <221> MISC\_FEATURE <222> (112)..(112) <223> Xaa = any amino acid <220> <221> MISC\_FEATURE <222> (113)..(113) <223> Xaa = any amino acid out of a mixture of Ala, Asp, Glu, Phe, Gly, Ile, Leu, Met, Pro, Gln, Ser, Thr, Val or Tyr

<220>

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<221> MISC\_FEATURE <222> (115)..(115) <223> Xaa = any amino acid out of a mixture of Phe, His, Ile, Leu, Asn, Pro, Ser, Val, Trp or Tyr <400> 236 Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu 10 Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Tyr Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile 35 Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys 50 Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu 65 Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala 85 100 Xaa Asp Xaa Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser 120 115 <210> 237 <211> 127 <212> PRT <213> artificial sequence <220> <223> synthetic construct; VH5 <220> <221> MISC FEATURE <222> (99)..(112)

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<223> Xaa = any amino acid out of a mixture of Ala, Asp, Glu, Phe, Gly, Ile, Leu, Met, Pro, Gln, Ser, Thr, Val or Tyr

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Gln Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Lys Pro Ser Gln 1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Ile Ser Gly Asp Ser Val Ser Ser Asn 20 25 30

Ser Ala Ala Trp Asn Trp Ile Arg Gln Ser Pro Gly Arg Gly Leu Glu
35 40 45

Trp Leu Gly Arg Thr Tyr Tyr Arg Ser Lys Trp Tyr Asn Asp Tyr Ala 50 60

Val Ser Val Lys Ser Arg Ile Thr Ile Asn Pro Asp Thr Ser Lys Asn 65 70 75 80

Gln Phe Ser Leu Gln Leu Asn Ser Val Thr Pro Glu Asp Thr Ala Val 85 90 95

Xaa Xaa Xaa Xaa Asp Xaa Trp Gly Gln Gly Thr Leu Val Thr Val 115 120 125

Ser Ser

130

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<220>
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<220>
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<222> (265)..(267)
<223> nnn = TTT, CAT, CTT, ATG or CAG
<220>
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<220>
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<222> (283)..(285)
<223> nnn = CTT, CCT or TCT
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<222> (280)..(282)
<223> nnn = GCT, GAT, GAG, TTT, GGT, CAT, ATT, AAG, CTT, ATG, AAT, CCT,
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<223> nnn = GCT, GAT, GGT, CAT, CTT, AAT or TCT
<220>
<221> misc feature
<222> (274)..(276)
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<220>

<221> misc feature

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<222>
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                                                                     60
attagctgca gaagcagcca aagcctgctg catagcaacg gctataacta tctggattgg
                                                                    120
                                                                    180
taccttcaaa aaccaggtca aagcccgcag ctattaattt atctgggcag caaccgtgcc
agtggggtcc cggatcgttt tagcggctct ggatccggca ccgattttac cctgaaaatt
                                                                    240
                                                                    300
agccqtqtqq aaqctqaaqa cgtgggcgtg tattattgcn cagnnnnnna cctttggcca
gggtacgaaa gttgaaatta aacgtacg
                                                                     328
<210> 241
<211> 330
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       CAG, CGT, TCT, ACT, GTT, TGG or TAT
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<223> nnn = can be ACT or GTT
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<221> misc feature
<222> (274)..(276)
<223> nnn = GCT, GAT, GAG, TTT, GGT, CAT, ATT, AAG, CTT, ATG, AAT, CAG,
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<222> (280)..(282)
<223> nnn = GCT, GAT, GGT, CAT, CTT, AAT or TCT
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      (283)..(285)
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<223> nnn = CTT, CCT or TCT
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                                                                       60
ctgagctgca gagcgagcca gagcgtgagc agcagctatc tggcgtggta ccagcagaaa
                                                                      120
ccaqqtcaaq caccgcgtct attaatttat ggcgcgagca gccgtgcaac tggggtcccg
                                                                      180
gcgcgtttta gcggctctgg atccggcacg gattttaccc tgaccattag cagcctggaa
                                                                      240
cctgaagact ttgcgnnnta ttattgcnnn cagnnnnnnn nnnnnnnnn nacctttggc
                                                                      300
cagggtacga aagttgaaat taaacgtacg
                                                                      330
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<211> 345
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<223> nnn = TTT, CAT, CTT, ATG or CAG
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attaactqca qaaqcaqcca qaqcqtqctq tataqcaqca acaacaaaaa ctatctggcg
                                                                     120
tggtaccage agaaaccagg teageegeeg aaactattaa tttattggge atecaccegt
                                                                     180
qaaaqcqqqq tcccqqatcq ttttaqcqqc tctggatccg gcactgattt taccctgacc
                                                                     240
atttcgtccc tgcaagctga agacgtggcg gtgtattatt gcnnncagnn nnnnnnnnn
                                                                     300
nnnnnacct ttggccaggg tacgaaagtt gaaattaaac gtacg
                                                                     345
<210> 243
<211>
       322
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<400> 243
gatatcgtgc tgacccagcc gccttcagtg agtggcgcac caggtcagcg tgtgaccatc
                                                                           60
tcgtgtagcg gcagcagcag caacattggc agcaactatg tgagctggta ccagcagttg *
cccgggacgg cgccgaaact gctgatttat gataacaacc agcgtccctc aggcgtgccg
                                                                          180
gatcgtttta gcggatccaa aagcggcacc agcgcgagcc ttgcgattac gggcctgcaa
                                                                          240
agcgaagacg aagcggatta ttattgccag tctngatnnn nnngtgtttg gcqqcgqcac
                                                                          300
                                                                          322
gaagttaacc gttcttggcc ag
<210> 244
<211> 336
<212> DNA
<213> artificial sequence
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<223> synthetic construct; VL lambda2
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<221> misc_feature
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CAG, CGT, TCT, ACT, GTT, TGG or TAT

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tttagcggat ccaacagcgg caacaccgcg accctgacca ttagcggcac tcaggcggaa	240
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tatagcacca gcctgaaaac gcgtctgacc attagcaaag atacttcgaa aaatcaggtg
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                                                                    180
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Glu 145	Gln	Leu	Lys	Ser	Gly 150	Thr	Ala	Ser	Val	Val 155	Cys	Leu	Leu	Asn	Asn 160
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Ser 225		Val	Thr	Lys	Ser 230		Asn	Arg	Gly	Glu 235		Met	Lys	Gln	Ser 240
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Gly	Ser	Leu 275	Arg	Leu	Ser	Cys	Ala 280	Ala	Ser	Gly	Phe	Thr 285	Phe	Ser	Ser
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Ile Ser Gln Thr Gly Arg Lys Ile Tyr Tyr Ala Asp Ser Val Lys Gly 50 55 60													
Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln 65 70 75 80													
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg 85 90 95													
Leu Thr His Tyr Ala Arg Tyr Tyr Arg Tyr Phe Asp Val Trp Gly Gln 100 105 110													
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Ile Ser Gln Thr Gly Arg Lys Ile Tyr Tyr Ala Asp Ser Val Lys Gly 50 55 60													
Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln 65 70 75 80													
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg 85 90 95													
Leu Thr His Tyr Ala Arg Tyr Tyr Arg Tyr Phe Asp Val Trp Gly Gln 100 105 110													
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60

120

180

240

300

360

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Leu Thr His Tyr Ala Arg Tyr Tyr Arg Tyr Phe Asp Val Trp Gly Gln

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg

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Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg 85 90 95													
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Gly Thr Leu Val Thr Val Ser Ser 115 120													
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Ile Ser Glu Ser Gly Lys Thr Lys Tyr Tyr Ala Asp Ser Val Lys Gly 50 55 60

Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln 65 70 75 80													
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg 85 90 95													
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Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ala 35 40 45	
Ile Asn Gly Thr Gly Met Lys Lys Tyr Tyr Ala Asp Ser Val Lys Gly 50 60	
Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln 65 70 75 80	
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg 85 90 95	
Gly Lys Gly Asn Thr His Lys Pro Tyr Gly Tyr Val Arg Tyr Phe Asp 100 105 110	
Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser 115 120	
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aagggtctcg agtgggtgag cgctattaat tataatggtg ctcgtattta ttatgctgat	180
tctgttaagg gtcgttttac catttcacgt gataattcga aaaacaccct gtatctgcaa	240
atgaacagcc tgcgtgcgga agatacggcc gtgtattatt gcgcgcgtgg taagggtaat	300
	360
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actcataagc cttatggtta tgttcgttat tttgatgttt ggggccaagg caccctggtg	372
	372

Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu

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15 5 10 1 Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met 25 30 20 Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ala 40 Ile Asn Tyr Asn Gly Ala Arg Ile Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln 75 70 Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg 90 Gly Lys Gly Asn Thr His Lys Pro Tyr Gly Tyr Val Arg Tyr Phe Asp 105 Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser 120 <210> 312 <211> 372 <212> DNA <213> artificial sequence <220> <223> synthetic construct <400> 312 caattggtgg aaagcggcgg cggcctggtg caaccgggcg gcagcctgcg tctgagctgc 60 geggeeteeg gatttacett tageagetat gegatgaget gggtgegeea ageecetggg 120 aagggtctcg agtgggtgag cgctattaat gctgatggta atcgtaagta ttatgctgat 180 tctgttaagg gtcgttttac catttcacgt gataattcga aaaacaccct gtatctgcaa 240 atgaacagcc tgcgtgcgga agatacggcc gtgtattatt gcgcgcgtgg taagggtaat 300 360 actcataaqc cttatqqtta tgttcqttat tttgatgttt ggggccaagg caccctggtg 372 acggttagct ca <210> 313 <211> 124 <212> PRT

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Arg	Leu	Ser	Cys 20	Ala	Ala	Ser	Gly	Phe 25	Thr	Phe	Ser	Ser	Tyr 30	Ala	Met	
Ser	Trp	Val 35	Arg	Gln	Ala	Pro	Gly 40	Lys	Gly	Leu	Glu	Trp 45	Val	Ser	Ala	
Ile	Asn 50	Ala	Asp	Gly	Asn	Arg 55	Lys	Tyr	Tyr	Ala	Asp 60	Ser	Val	Lys	Gly	
Arg 65	Phe	Thr	Ile	Ser	Arg 70	Asp	Asn	Ser	Lys	Asn 75	Thr	Leu	Tyr	Leu	Gln 80	
Met	Asn	Ser	Leu	Arg 85	Ala	Glu	Asp	Thr	Ala 90	Val	Tyr	Tyr	Cys	Ala 95	Arg	
Gly	Lys	Gly	Asn 100	Thr	His	Lys	Pro	Tyr 105	Gly	Tyr	Val	Arg	Tyr 110		Asp	,
Val	Trp	Gly 115	Gln	Gly	Thr	Leu	Val 120		Val	Ser	Ser					
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aaç	ggto	ctcg	agtg	ggtg	ag c	gcta	ıttaa	ıt gc	tgat	ggta	ato	gtaa	igta	ttat	gctgat	180
tct	gtta	aagg	gtcg	rtttt	ac c	attt	caco	ıt ga	ıtaat	tcga	aaa	acac	ecct	gtat	ctgcaa	240
ato	gaaca	agcc	tgcg	ıtgag	ıga a	gata	cggc	c gt	gtat	tatt	gag	gagag	ıtgg	taac	ggtaat	300
act	cata	aaqc	ctta	ıtggt	ta t	gtto	gtta	at tt	tgat	gttt	ggg	gcca	agg	caco	cctggtg	360

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Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ala 35 40 45													
Ile Asn Ala Asp Gly Asn Arg Lys Tyr Tyr Ala Asp Ser Val Lys Gly 50 60													
Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln 65 70 75 80													
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg 85 90 95													
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atgaacagcc tgcgtgcgga agatacggcc gtgtattatt gcgcgcgtgg taag	gggtaat 300
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Ile Asn Ala Asn Gly Tyr Lys Lys Tyr Tyr Ala Asp Ser Val Ly 50 55 60	s Gly
Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Le 65 70 75	u Gln 80
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Al 85 90	a Arg
Gly Lys Gly Asn Thr His Lys Pro Tyr Gly Tyr Val Arg Tyr Ph	ne Asp
Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser 115 120	
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atgaacagcc tgcgtgcgga agatacggcc gtgtattatt gcgcgcgtgg taagggtaat	300
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Ile Asn Ala Asn Gly Tyr Lys Lys Tyr Tyr Ala Asp Ser Val Lys Gly 50 55 60	
Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln 65 70 75 80	
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg 85 90 95	
Gly Lys Gly Asn Thr His Lys Pro Tyr Gly Tyr Val Arg Tyr Phe Asp 100 105 110	
Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser 115 120	

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aagg	gtct	cg a	gtgg	gtga	g cg	ctat	taat	gct	aatg	gtt	ataa	gaag	ta t	tatg	ctgat	180	
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actc	ataa	gc c	ttat	ggtt	a tg	ttcg	ttat	ttt	gatg	ttt	aaaa	ccaa	gg c	accc	tggtg	360	
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Arg	Leu	Ser	Cys 20	Ala	Ala	Ser	Gly	Phe 25	Thr	Phe	Ser	Ser	Tyr 30	Ala	Met		
Ser	Trp	Val 35	Arg	Gln	Ala	Pro	Gly 40	Lys	Gly	Leu	Glu	Trp 45	Val	Ser	Ala		
Ile	Asn 50	Ala	Asn	Gly	Tyr	Lys 55	Lys	Tyr	Tyr	Ala	Asp 60	Ser	Val	Lys	Gly		
Arg 65	Phe	Thr	Ile	Ser	Arg 70	Asp	Asn	Ser	Lys	Asn 75	Thr	Leu	Tyr	Leu	Gln 80		
Met	Asn	Ser	Leu	Arg 85	Ala	Glu	Asp	Thr	Ala 90	Val	Tyr	Tyr	Cys	Ala 95	Arg		
Gly	Lys	Gly	Asn	Thr	His	Lys	Pro	Tyr	Gly	Tyr	Val	Arg	Тух	Phe	Asp		

110

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105

100

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Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg 85 90 95	
Leu Leu Ser Arg Gly Tyr Asn Gly Tyr Tyr His Lys Phe Asp Val Trp 100 105 110	
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ccaggtcaag caccgcgtct attaatttat ggcgcgagca gccgtgcaac tggggtcccg	180
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Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Val Pro Ala Arg Phe Ser

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60 50 55 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu 70 Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Thr Tyr Asp Tyr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr 105 100 <210> 326 <211> 330 <212> DNA <213> artificial sequence <220> <223> synthetic construct <400> 326 gatategtge tgacecagag eeeggegace etgageetgt eteegggega aegtgegace 60 120 ctgagctgca gagcgagccg gcgtattcat gtttattatc tggcgtggta ccagcagaaa ccaggtcaag caccgcgtct attaatttat ggcgcgagca gccgtgcaac tggggtcccg 180 gcgcgtttta gcggctctgg atccggcacg gattttaccc tgaccattag cagcctggaa 240 cctgaagact ttgcgactta ttattgccag cagacttatg attatcctcc tacctttggc 300 330 cagggtacga aagttgaaat taaacgtacg <210> 327 <211> 110 <212> PRT <213> artificial sequence <220> <223> synthetic construct <400> 327 Asp Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Arg Arg Ile His Val Tyr Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu 40

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Val Pro Ala Arg Phe Ser 50 60	
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu 65 70 75 80	
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Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Val Pro Ala Arg Phe Ser 50 60	
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu 65 70 75 80	
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Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Arg Arg Ile His Val Tyr 20 25 30	
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu 35 40 45	

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Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Val Pro Ala Arg Phe Ser

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu 65 70 75 80

Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Thr Tyr Asp Tyr Pro 85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr 100 105 110

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Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Arg Arg Ile His Val Tyr 20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu

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40 45 35 Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Val Pro Ala Arg Phe Ser 50 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu 70 Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Thr Tyr Asp Tyr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr 105 100 <210> 334 <211> 330 <212> DNA <213> artificial sequence <220> <223> synthetic construct <400> 334 qatatcqtqc tqacccagaq cccggcgacc ctgagcctgt ctccgggcga acgtgcgacc 60 ctgagctgca gagcgagcca gcgtcttggt cgtctttatc tggcgtggta ccagcagaaa 120 ccaqqtcaaq caccqcqtct attaatttat qqcqcqaqca qccqtqcaac tqqqqtcccq 180 gcgcgtttta gcggctctgg atccggcacg gattttaccc tgaccattag cagcctggaa 240 cctgaagact ttgcgactta ttattgccag cagacttatg attatcctcc tacctttggc 300 330 cagggtacga aagttgaaat taaacgtacg <210> 335 <211> 110 <212> PRT <213> artificial sequence <220> <223> synthetic construct <400> 335 Asp Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Arg Leu Gly Arg Leu 25

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Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Val Pro Ala Arg Phe Ser 50 55 60	
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu 65 70 75 80	
Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Thr Tyr Asp Tyr Pro 85 90 95	
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Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Phe Ile Gln Arg Phe 20 25 30	

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Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu 40 Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu 70 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Thr Tyr Asn Tyr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr 105 <210> 338 <211> 330 <212> DNA <213> artificial sequence <220> <223> synthetic construct <400> 338 gatategtge tgacecagag eceggegace etgageetgt eteegggega aegtgegace 60 ctgagctgca gagcgagcca gtatgttgat cgtacttatc tggcgtggta ccagcagaaa 120 ccaggtcaag caccgcgtct attaatttat ggcgcgagca gccgtgcaac tggggtcccq 180 gcgcgtttta gcggctctgg atccggcacg gattttaccc tgaccattag cagcctggaa 240 cctgaagact ttgcgactta ttattgccag cagatttatt cttttcctca tacctttgqc 300 cagggtacga aagttgaaat taaacgtacg 330 <210> 339 <211> 110 <212> PRT <213> artificial sequence <220> <223> synthetic construct <400> 339 Asp Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Tyr Val Asp Arg Thr

25

20

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Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Arg Phe Phe Tyr Lys

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20 25 30 Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Ser Gly Ser Ser Asn Arg Ala Thr Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu 70 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Leu Gln Leu Tyr Asn Ile Pro Asn Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr 100 105 <210> 342 <211> 330 <212> DNA <213> artificial sequence <220> <223> synthetic construct <400> 342 gatategtge tgacecagag ceeggegace etgageetgt eteegggega aegtgegace 60 ctgagctgca gagcgagcca gtatgttgat cgtacttatc tggcqtqqta ccaqcaqaaa 120 ccaggtcaag caccgcgtct attaatttat qqcqcqaqca qccqtqcaac tqqqqtcccq 180 gcgcgtttta gcggctctgg atccggcacg gattttaccc tgaccattag cagcctggaa 240 cctgaagact ttgcgactta ttattgccag cagatttatt cttttcctca tacctttggc 300 cagggtacga aagttgaaat taaacgtacg 330 <210> 343 <211> 110 <212> PRT <213> artificial sequence <220> <223> synthetic construct <400> 343 Asp Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly 10

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Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Val Pro Ala Arg Phe Ser 50 60
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu 65 70 75 80
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His Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr 100 105 110
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20 25 30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu 35 40 45

Ile Ser Gly Ser Ser Asn Arg Ala Thr Gly Val Pro Ala Arg Phe Ser 50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu 65 70 75 80

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ecaggteaag eacegegtet attaatttat ggegegagea geegtgeaae tggggteeeg 180
gegegttta geggetetgg ateeggeaeg gattttaeee tgaccattag eageetggaa 240
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Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Val Pro Ala Arg Phe Ser 50 60										
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu 65 70 75 80										
Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ile Tyr Ser Phe Pro 85 90 95										
His Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr 100 105 110										
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Asp Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly

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Tyr Le	u Ala 35	Trp	Tyr	Gln	Gln	Lys 40	Pro	Gly	Gln	Ala	Pro 45	Arg	Leu	Leu		
Ile Se 50	r Gly	Ser	Ser	Asn	Arg 55	Ala	Thr	Gly	Val	Pro 60	Ala	Arg	Phe	Ser		
Gly Se	r Gly	Ser	Gly	Thr 70	Asp	Phe	Thr	Leu	Thr 75	Ile	Ser	Ser	Leu	Glu 80		
Pro Gl	u Asp	Phe	Ala 85	Val	Tyr	Tyr	Cys	Leu 90	Gln	Leu	Tyr	Asn	Ile 95	Pro		
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ccaggt	caag	cacc	gcgt	ct at	taat	ttat	t ggo	zgaga	agca	gcc	gtgca	aac 1	tggg	gtcccg	180	)
gcgcgt															240	)
cctgaa	gact	ttgc	gacti	ta ti	att	gcca	g caq	gctti	tctt	ctta	atcct	ca t	tacct	ttggc	306	Э
cagggt	acga	aagt <sup>.</sup>	tgaa	at ta	aaac	gtac	3								330	Э
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